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DOMINANT VS. RECESSIVE LETHAL MUTATION

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The purpose of this article is to call attention to three circumstances under which the sensitivity of chromosomes, in sperm of *Drosophila melanogaster*, to the induction by x-rays of recessive lethal mutations appears to have been altered in one direction while their sensitivity to induction of dominant lethal mutations has been simultaneously altered in the opposite direction.

The first circumstance concerns a difference of strain. Demerec, Kaufmann, and Hoover[1] have reported that an Oregon-R strain exhibits both a lower recessive lethal mutation rate and a higher dominant lethal mutation rate than the Swedish-b strain.

The second circumstance involves the age of males at the time of irradiation: Hansen and Heys,[2] in an experiment which seems to have attracted remarkably little attention, report that the sensitivity of the chromosomes in the sperm to the induction of recessive lethal mutations decreases fairly rapidly with the age of males at time of irradiation. The writer has found the opposite effect for dominant lethal mutations (as measured by proportion of eggs hatching from females fertilized by irradiated males), the survival, for a dose of 2300 r units, being about 60% where the males are 1 day old and about 42% where the males are 16 days old. Eighteen pairs of flies in four age groups were tested and the chi square for variation within all age groups is 5.65 with 14 degrees of freedom (P = 0.95–0.98) and between age groups is 17.67 with 3 degrees of freedom (P less than 0.001). The chi square within age groups seems suspiciously high but this is probably a matter of chance since in a number of dominant lethal experiments performed here the chi squares within groups have shown reasonable agreement with values expected in random sampling. It should perhaps be emphasized that in the experiments of Hansen and Heys as well as in those reported above the males were isolated before copulation.

The third circumstance involves differential effects of neutrons and

x-rays in the production of mutations of the two categories. Using a physical estimate of the number of ions per cc., the uncertainty of which would not affect the relative results, neutron-produced ions were found to be about 0.75 times as efficient as x-ray-produced ions in the induction of recessive sex-linked lethal mutations (which is in reasonable agreement with the results of Timofeeff-Ressovsky and Zimmer,[3] considering the differences in ionization-measuring instruments), and about 1.5 times as efficient in the production of dominant lethals, where doses of x-rays and neutrons are applied which result in the death of 50% of the F_1 eggs.

It may be significant that the efficiency of neutron-produced ions in the induction of gross (genetically detected) translocations appears to be intermediate, compared to x-rays, to their efficiencies in the production of recessive and dominant lethals. The factor obtained (approximately 1.25) applies for a dose equivalent to 2500 r units of x-rays.

The data obtained are not critical for determining whether there may be some differences in the general dosage-aberration relationships for neutrons, as compared to x-rays; such a difference has been reported by Giles[4] for the case of exchange breaks in *Tradescantia* microspores.

[1] *Carnegie Inst. of Washington, Yr. Bk.*, No. 37, 40–47 (1938).
[2] *Amer. Nat.*, **68**, 166–167 (1934).
[3] *Naturwiss.*, **26**, 21–22, 362–365 (1938).
[4] *Proc. Nat. Acad. Sci.*, **26**, 567–575 (1940).

LINKAGE STUDIES OF THE RAT (RATTUS NORVEGICUS) IV

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A new mutation of the rat, "wobbly," was first observed in September, 1933, in the laboratory of Professor Amy L. Daniels at the University of Iowa, in the course of an experiment in which the effects of variation in the manganese content of the diet were being studied. This mutation is a simple recessive in inheritance and is characterized by a peculiarity of locomotion of affected individuals first observable at about the time the eyes open at the age of 14 days or earlier, locomotion being jerky instead of the normal steady gliding movement of the young when taken from the nest and placed on a table. The young wobbly individual may stand in a hesitating attitude with head and particularly tail elevated, before it begins to crawl. Then it progresses a step at a time with a slight jerk of the body first forward then backward as each step is taken. The contrast is

very striking to the steady, smooth locomotion of normal animals, with head and tail horizontal. The peculiar behavior of the wobbly rats was at first thought to be an indication of manganese poisoning but this explanation was abandoned when repetition of the experiment failed to reproduce the wobbly symptoms.

Dr. Daniels states that the animals used in the first experiment were descendants of the Wisconsin stock which had been inbred in her laboratories here in Iowa and in Wisconsin since 1914. Inquiry of Professor Steenbock regarding the development of any such abnormality among his animals which were of the same original stock threw no light on the subject. None of his rats had ever shown such symptoms. Dr. Ingram, the neurotomist who saw these animals, thought the condition was the result of an infection of the inner ear. Histologic sections, however, showed no abnormality. This is a finding similar to that made by several investigators in the case of the Japanese waltzing mouse.

In repeating the experiment new uncontaminated animals were fed during four generations various levels of manganese (from 1.5 mg. to 30 mg. per rat per day) as manganese sulphate. The manganese sulphate in solution was added to a small amount of milk and fed apart from the stock ration, each animal thus consuming his allotted portion. Neither during the year nor subsequently have there appeared any rats with these queer symptoms.

Having failed to reproduce the wobbly symptoms by manganese and manganese-fluorine diets, Dr. Daniels made a reëxamination of her records and found that a certain number of rats from the stock colony had been used in a comparative study of bone development in rats and human infants, in the course of which the rats had been subjected to x-rays, and later restored to the stock colony. It is possible but not certain that one or more x-rayed individuals may have been among the ancestors of wobblies, but even if this were true, it is doubtful whether the x-raying can reasonably be regarded as the causative agent, since spontaneous mutations affecting nervous behavior (waltzing in rats, mice, and guinea-pigs, "shaker" behavior in mice) have occurred without x-ray treatment.

When Dr. Daniels came to the conclusion that the wobbly state was due to a genetic mutation, however caused, she offered generously to share her material with the co-authors for a study of its linkage relations with other known genes, of which they possessed a nearly complete assortment. Accordingly wobbly stocks were established in Berkeley and Philadelphia and a comprehensive program of linkage tests was outlined.

At that time two linkage systems had been established for the rat, as follows. Chromosome I was known to contain five mutant genes, in the order *l c r p w*. Chromosome II was known to contain two mutant genes, *Cu* and *b*, but while this investigation was in progress a third mutant gene

an was found to lie in Chromosome II, the order of the three genes being *Cu an b*. Seven other mutant genes had been shown to be independent of genes in Chromosomes I and II and of one another, thus being markers of as many independent chromosomes. They are genes *a*, Cu_2, *d*, *h*, *hr*, *j* and *k*.

To test the linkage relations of wobbly with one or more genes from each of the linkage systems and with each of the seven independent genes, the following crosses were made:

I. 1. Wobbly (*wo*) × pink-eye (*p*);
   1ª. Wobbly (*wo*) × albino (*c*);
II. 2. Wobbly (*wo*) × anemia (*an*);
   2ª. Wobbly (*wo*) × curly (*Cu*);
   3. Wobbly (*wo*) × agouti (*A*);
   4. Wobbly (*wo*) × curly$_2$ (Cu_2);
   5. Wobbly (*wo*) × dilution (*d*);
   6. Wobbly (*wo*) × hooded (*h*);
   7. Wobbly (*wo*) × hairless (*hr*);
   8. Wobbly (*wo*) × jaundiced (*j*);
   9. Wobbly (*wo*) × kinky (*k*).

Some of these crosses were made in combination, as, for example, 3 and 6, the original wobbly stock being homozygous for black hooded (*aa hh*). All data concerning the same gene from whatever cross obtained have been combined in a single total, as recorded in the tables.

Seven of the test crosses were made in Berkeley and four (1ª, 2ª, 7 and 9) were made in Philadelphia. From six of the eleven crosses F_1 animals were back-crossed to double recessive individuals, from which sort of matings approximately equal numbers of crossover and non-crossover combinations are expected, if no linkage exists. Such a result was actually obtained, as shown by the data included in table 1.

TABLE 1

BACK-CROSS DATA ON THE LINKAGE RELATION OF WOBBLY TO SIX OTHER MUTANT GENES

CROSS	GENE	NON-CROSSOVERS	CROSS-OVERS	DEVIATION	P.E.	DEV./P.E.	OBSERVER
1	*p*	93	97	2.0	4.6	0.43	Castle
2ª	*Cu*	24	25	0.5	2.3	0.02	King
3	*a*	45	37	4.0	3.0	1.30	Castle
4	Cu_2	70	70	0	4.0	0	Castle
5	*d*	103	107	2.0	4.9	0.41	Castle
6	*h*	211	188	11.5	6.7	1.70	Castle

For the four remaining crosses (2, 7, 8 and 9) backcrosses of the ordinary type were not possible since double recessive individuals either could not be obtained or were very feeble because of the lethal or enfeebling character of

the combination. Accordingly a method outlined by Castle in 1939 was followed. F_1 was mated to animals carrying neither of the two genes involved in the cross. The progeny of such matings were tested individually for presence of each of the two genes contributed to F_1 in the repulsion relationship. The tests were made by mating the individual to be tested to an ordinary F_1 individual. A litter of six or more young was considered a conclusive test. The animals tested fall into four classes, viz. (1) carriers of both mutant genes, (2) carriers of neither, (3) carriers of one gene only and (4) carriers of the other gene only. Classes (1) and (2) would have received crossover combinations from the F_1 parent; classes (3) and (4) would have received non-crossover (repulsion) combinations from the F_1 parent. From table 2 it will be seen that in crosses 2, 8 and 9, no significant

TABLE 2

DATA ON THE LINKAGE RELATION OF WOBBLY TO FOUR OTHER MUTANT GENES, OBTAINED BY THE "LETHAL TEST" METHOD

CROSS	GENE	NON-CROSSOVERS	CROSS-OVERS	DEVIATION	P.E.	DEV./P.E.	OBSERVER
2	*an*	82	79	1.5	4.3	0.35	Castle
7	*hr*	71	48	11.5	3.68	3.12	King
8	*j*	72	71	0.5	4.0	0.12	Castle
9	*k*	65	66	0.5	3.8	0.13	King

difference is found between the totals for crossover and non-crossover combinations, respectively, but in cross 7 a significant difference is found, non-crossovers being in excess, showing repulsion between wobbly and hairless, which establishes their location in the same chromosome (which we shall designate Chromosome III) with an indicated loose linkage between the two genes of 40.33 ± 3.09 per cent, which is very similar in amount to the linkage in Chromosome II, between genes *Cu* and *b*, which was found by King and Castle (1935) to be 40.48 ± 1.35.

In testing for linkage between hairless and wobbly (cross 7) by the lethal test method, Dr. King mated 119 outcross individuals to F_1 animals. Of these

Non-crossovers { 32 produced normal and wobbly young;
39 produced normal and hairless young;
Crossovers { 20 produced normal and both wobbly and hairless young;
28 produced normal but neither wobbly nor hairless young.

The odds are 27 to one against the occurrence of so great an inequality as this between crossover and non-crossover classes by chance alone.

Supplementing cross 1, and confirming the finding from the cross with pink-eye that wobbly does not lie in Chromosome I, a cross (1^a) was made

by King between wobbly and albino (*c*). An F_2 population of 186 young consisted of the following classes:

	C Wo	*C wo*	*c Wo*	*c wo*
Observed	87	41	45	13
Expected	104.6	34.9	34.9	11.6

No indication of linkage is given by this population, since the critical recombination class *c wo* which could arise only from crossover gametes is even slightly greater than expected.

Summary—The mutant gene "wobbly" discovered by Daniels has been shown by linkage tests made by King and Castle to lie in the same chromosome (III) as hairless, with a crossover percentage of 40.3 ± 3.1.

[1] King, Helen Dean, and Castle, W. E., "Linkage Studies of the Rat," *Proc. Nat. Acad. Sci.*, **21**, 390–399 (1935).

[2] King, Helen Dean, and Castle, W. E., "Linkage Studies of the Rat, II," *Ibid.*, **23**, 56–60 (1937).

[3] Castle, W. E., and King, Helen Dean, "Linkage Studies of the Rat, III," *Ibid.*, **26**, 578–580 (1940).

[4] Castle, W. E., "On a Method for Testing for Linkage between Lethal Genes," *Ibid.*, **25**, 593–594 (1939).

[5] Grüneberg, H., "Linkage Relations of a New Lethal Gene in the Rat," *Genetics*, **24**, 732–746 (1939).

THE EFFECT OF ADULT BODY COLOR MUTATIONS UPON THE LARVA OF DROSOPHILA MELANOGASTER

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In an organism widely used, as is *Drosophila melanogaster*, for genetical, cytological and embryological experimentation, the effects brought about during development by those mutations which alter the adult phenotype are of importance from both the theoretical and the practical standpoints. From the theoretical point of view, the genetic factors whose effects are recognizable during embryogeny offer rich material for the study of the mechanism of gene action, since observations upon the early action of a gene may provide information which is important in understanding its effect upon the adult. From a utilitarian standpoint, mutants which can be identified during the larval period are useful in classifying the larvae required for salivary chromosome preparations, for transplantation experi-

ments and for studies of physiology and growth. The color of the larval Malpighian tubes of certain eye-color mutants and that of the mouth armature of larvae homozygous for the body color mutation, yellow, is available for use in this manner. Other genetic factors which affect the larva, such as chubby, giant, vestigial and the Minutes, cannot be used for classification because the expression of the mutant character overlaps the wild type or because penetrance is low.

It was found by Brehme[1] in 1937 that the cephalopharyngeal skeleton or "jaws" of the larva homozygous for yellow are lighter in color at all stages from hatching to pupation. The wild-type mouthparts are dark brown; those of the yellow larva are golden in the first instar, and golden brown at the basal region during the second and third instars. Classification of the living larva can readily be made at all stages by means of this difference. Kaliss,[2] in an embryological study of a deficiency, *Df*(1)260–2, in which the yellow and achaete loci are lacking, found that the larvae which are hemizygous for the deficiency and die in the late embryonic stage have yellow mouthparts. In view of these two cases, it has seemed desirable to examine the yellow alleles and other body and bristle color mutants for their effect upon the larval stage.

Methods.—Mass matings were made of each stock under consideration, and eggs were collected over a 24-hour period by a modification of the bottle-cap method of Schweitzer,[3] using a standard banana-agar. The medium on which the eggs had been laid was then placed in Petri dishes containing banana-agar seeded with live yeast suspension. As preliminary observations showed no difference in the color of the mouthparts of optimally fed and of small, inadequately fed larvae, no attempt was made subsequently to control the number of larvae in a culture. All experimental material was maintained at 25°C.

The larvae were examined under a binocular microscope, against a white background. Observations were facilitated when the larvae were immersed in water. Both living and dissected larvae were examined, and permanent mounts in balsam were subsequently made of the mouthparts.

At the moults which signalize the end of the first and second instars, the mouthparts are shed with the hypodermis. Previous to the moult, new mouthparts are formed by hypodermal tissue and underlie the older jaws until the latter are shed. The new mouthparts are colorless from the time of their appearance until the moult; they then darken within a few minutes. In observing the color of the larval jaws, it is therefore essential to examine larvae in which this process of darkening has been completed. For this reason larvae can be studied throughout the first instar but only during the late second and third instars.

Results.—All available mutants were examined which affect adult body or bristle color, and each was compared with larvae from the Florida or

Swedish-*b* wild-type stocks. The larval mouthparts of the wild type are heavily pigmented with what is generally conceded to be melanin. The mouth hooks, mentum and anterior part of the vertical plate (Fig. 1) are very dark brown, almost black; the posterior, basal prongs are less pigmented than the rest and appear golden brown at the tips.

The results are summarized in table 1. Of the mutations which darken adult body color (black, ebony, sable, dusky and speck) only the ebony alleles darken the color of the larval mouthparts. The difference between the ebony and wild-type larvae is not great and is not useful for classification. This result is to be expected, since the wild-type jaws are already very heavily pigmented. It was, however, observed that the sclerotization around the posterior spiracles, which is golden in the wild type, is dark in the ebony mutants at all stages of the larval period. The anterior spiracles, which are sclerotized only in the third instar, are similarly darkened at this time. This darkened color of the spiracle sheaths characterizes the alleles ebony, ebony[4] and ebony[11], and is visible, although the difference is less great, in ebony-sooty. This larval character can be used for classification as early as hatching from the egg. No difference from the wild type in color of the spiracle sheath has been observed in any other mutant.

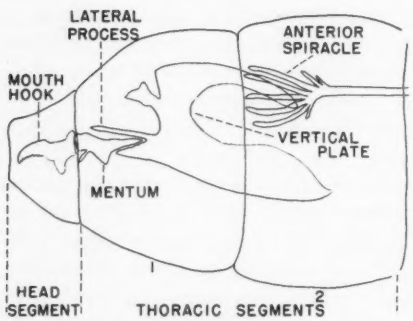

FIGURE 1

Mouthparts of the third instar larva of *D. melanogaster*, adapted from Sturtevant.[4] Lateral view; all parts shown in the diagram are paired.

Certain of the mutations which lighten the body or bristle color of the adult do not affect the larval mouthparts—silver, Blond translocation, straw, straw[4] and yellow[34c]. In the majority of cases, however, lightened pigmentation of the adult is paralleled by a lighter color of the larval jaws; the tan alleles, straw[2] and straw,[3] and all the yellow alleles except yellow[34c] produce this effect. The greatest difference from the wild type is seen in the cases of yellow[35a] and yellow[260-28], although it cannot be said that the adults of these genotypes are markedly lighter in color than the other extreme yellow alleles, such as yellow or yellow.[4] One case of factor interaction was studied: the yellow-silver combination, in which the flies are lighter in body color than either yellow or silver, shows a corresponding difference in color of the larval mouthparts, which are lighter than those of yellow.

In general, it may be said that where the difference in color of mutant and wild-type larval jaws is great, classification is possible as early as hatching from the egg; where the difference is slight, only third instar jaws can be classified in the living larva, and in certain cases, classification is reliable only when the mouthparts are dissected from the larva. No sex difference has been observed in the color of the larval mouthparts of any genotype. Although a large number of eye color mutants and other stocks have been examined, no effect on color of the larval jaws has ever been found except in those cases listed in table 1.

Three of the stocks studied, yellow^{31c}, yellow ^{3P} and duplication 260-25*b*, show mottling for yellow in the adult. No indication of mottling was found in the larval mouthparts.

In examining the Malpighian tube color of the larvae of certain eye color mutants, Beadle[5] found one case of maternal or premeiotic effect; homozygous light larvae have colorless tubes if they are the offspring of homozygous light females, wild-type (yellow) tubes if the mothers are heterozygous for light. Each of the yellow alleles listed in table 1 were tested for such an effect by outcrossing to the Swedish-*b* wild-type stock and subsequent backcrossing to yellow; the larvae of the backcross generation segregated for yellow in the expected 1:1 ratio and the color of the larval jaws of the yellow segregants were in all cases indistinguishable from those of the parent yellow stock. The color of the larval mouthparts of the yellow alleles, therefore, does not show a maternal effect.

Discussion.—Melanin production is the end-result of a series of chemical reactions which involve tyrosinase, oxygen and a chromogen, and the amount of melanin produced may be affected through an effect upon any one of these substances, as well as through other factors which influence the reaction. The only quantitative study which has been made of the melanin-producing system in Drosophila is that of Graubard,[6] who measured the relative quantities of extractable tyrosinase in larvae and pupae of the wild-type, yellow, black and ebony genotypes. He found that yellow larvae and pupae do not contain less tyrosinase than the more heavily pigmented stocks; in fact, in the pupal period, the relative tyrosinase content may be indicated as: $y > + > b > e$. By different methods of extraction, he was led to conclude that inhibitors of tyrosinase activity are present in both larval and pupal stages, and that the production of melanin is not so much a question of the presence or absence of the enzyme or its quantity as of an internal environment which allows the enzyme to take part in the reaction. No study has been made of the oxygen consumption of the larvae and pupae of the body color mutants, nor of the nature of the chromogen involved in melanin production. The body color mutants may therefore have their effect on pigmentation through inhibitors of tyrosinase activity, through the rate of oxygen uptake, through some aspect of the

TABLE 1

THE EXPRESSION IN THE ADULT AND LARVAL STAGES OF MUTATIONS AFFECTING PIGMENTATION

LOCUS	MUTANT	ADULT BODY COLOR	ADULT BRISTLE COLOR	LARVAL SPIRACLE COLOR	LARVAL MOUTHPARTS	POSSIBILITY OF CLASSIFICATION IN LARVAL STAGE
black	*b*	Dark	+	+	+	Not classifiable
ebony	*e*	Very dark	+	Dark, sooty	Darker than +; almost no brown showing	Classifiable by spiracle sheath
	e^4	Very dark	+	Dark, sooty	Like *e*	Like *e*
	e^{11}	Very dark	+	Dark, sooty	Like *e*	Like *e*
	e^5	Dark	+	Slightly dark	Like *e*	Like *e*
sable	*s*	Dark	+	+	+	Not classifiable
	s^2	Dark	+	+	Probably +	Not classifiable
dusky	*dy*	+ but wings dark	+	+	+	Not classifiable
speck	*sp*	Dark	+	+	+	Not classifiable
	sp^2	Dark	+	+	+	Not classifiable
tan	*t*	Yellower than +, antennae light	+	+	Basal prongs light, difference slight	Difficult in dissected larva
	t^2	Slightly darker than *t*	+	+	Like *t*	Like *t*
	t^3	Lighter than +	+	+	Like *t*	Like *t*
suppressor of tan	*su-t t*	+	+	+	+	Not classifiable
straw	*stw*	Probably +	Light at tips, hairs yellowish	+	May be slightly lighter than + at prongs	Not classifiable
	stw^2	Yellowish	Brownish with light tips, hairs yellowish	+	Basal prongs strawish	Difficult in living larva
	stw^3	Yellowish	Hairs and bristles straw yellow	+	Basal prongs straw, vertical plate light	Classifiable in living larva
	stw^4	Very pale	Nearly black	+	+	Not classifiable
silver	*svr*	Pale, silvery	Dark	+	+	Not classifiable
Blond	*T*(1; 2)*Bld*	+	Yellow tips	+	+	Not classifiable
yellow	*y*	Yellow	Brown, yellow tips	+	Mouth hooks dark, rest brown, prongs golden	Classifiable in living larva
	y^2	Yellow	Black	+	Basal prongs lighter, rest dark	Not classifiable
	y^3	Tannish yellow	Black or nearly so	+	Basal prongs golden, lateral process and hooks light	Difficult in living larva
	y^4	Yellow	Brown, yellow tips	+	Mouth hooks dark, rest brown, prongs golden	Classifiable in living larva
	y^6	Yellow	Brown, yellow tips	+	Mouth hooks dark, rest	Classifiable in

	y^{6}	Yellow	Brown, yellow tips	+	Mouth hooks dark, rest brown, prongs golden	Classifiable in living larva
	$y^{1.8}$	Yellow	Brown, yellow tips	+	Mouth hooks dark, rest golden brown	Classifiable in living larva
	$y^{2.8}$	Tanner than *y*	Not quite black	+	Mouth hooks and mentum dark, rest golden brown	Classifiable in living larva
	$y^{3.P}$	Tannish	Black	+	Basal prongs more golden, rest dark	Difficult in living larva
	$y^{3.d}$	Tannish yellow	Black or nearly so	+	Golden brown, mouth hooks light	Classifiable in living larva
	$y^{4.d}$	Tan, antennae light	Brown	+	Golden brown, mouth hooks dark	Classifiable in living larva
	$y^{7.2}$	Tan	Black	+	Basal prongs slightly lighter than +, rest dark	Not classifiable
	$y^{34.c}$	Tannish	Black	+	+	Not classifiable
	$y^{35.a}$	Yellow	Brown, gold tips	+	Golden, mouth hooks very light	Classifiable in living larva
	$y^{31.b}$	Yellow	Brown, gold tips	+	Base golden, mentum light, mouth hooks slightly light	Classifiable in living larva
	$y^{31.c}$	Yellow	Black, mutating to *y ac*	+	Basal prongs light, mouth hooks dark	Difficult in living larva
	$y^{31.d}$	Yellow	Black	+	Basal prongs light, mouth hooks dark	Difficult in living larva
	y^{260-28}	Yellow	Golden	+	Basal prongs light, rest dark	Difficult in living larva
Deficiency affecting yellow*	*Df*(1)260-10	Yellow	Light	+	Base very light, vertical plate light, mouth hooks slightly light	Classifiable in living larva
yellow duplication†	*Dp*(1)260-25*b*	Yellow brown	Gold tips, mottling	+	Basal prongs light brown, rest dark	Difficult in living larva
yellow deficency	*Df*(1)260-2	Dies in embryonic stage			Yellow (Kaliss[2])	
yellow silver		Very light	Yellow	+	Lighter than *y*	Classifiable in living larva

* *Df*(1)260-10 lacks bands 1*A*1-2 of the salivary chromosome; the yellow locus, which is not included in the deficiency, is modified through a position effect to produce the yellow phenotype.

† The stock of *Dp*(1)260-25*b* is homozygous for the yellow mutation and also for a duplication carrying the wild-type alleles of *y* and *ac* inserted at the right of *bb* and causing mottling for yellow.

chromogen or through changes in the internal environment of the larva or pupa.

Graubard found by extraction of tyrosinase with chloroform that larvae contain the enzyme and a substrate, pupae only the enzyme, while no enzyme is present in the fly. It is thus probable that at least the early steps of formation of adult pigment take place in the larval and early pupal stages. It is not, therefore, surprising that genetically controlled differences in adult pigmentation are frequently paralleled by differences in larval pigmentation.

Certain of the mutations which lighten pigmentation affect three characters, body color, bristle color and color of the larval mouthparts. Others affect only two of the characters, while several mutants affect only one. The following relations may be derived from table 1:

Body color, bristles and larval mouthparts affected: *stw*^{2}, *stw*^{3}, *y*, *y*^{4}, *y*^{6}, *y*^{1S}, *y*^{2S}, *y*^{td}, *y*^{35a}, *y*^{31b}, *y*$^{260\text{-}28}$, *Dp*(1)260-25*b*, *Df*(1)260-10.
Body color and bristles affected, not larval mouthparts: none.
Body color and larval mouthparts affected: *t*, *t*^{2}, *t*^{3}, *y*^{2}, *y*^{3}, *y*^{3P}, *y*^{3d}, *y*^{v2}, *y*^{31c}, *y*^{31d}.
Body color only affected: *stw*^{4}, *svr*, *y*^{34c}.
Bristles and larval mouthparts affected: *stw* (slight effect).
Bristles only affected: Blond.
Larval mouthparts only affected: none.

These relations indicate that when the processes of melanin production are sufficiently upset to affect both adult body and bristle color, the effect begins early and influences larval melanin also; an effect on body color but not on bristle color may also be accompanied by an effect on larval melanin. In most cases, a considerable upset appears necessary to influence the deposition of the pigment as early as the larval stage. It would seem, however, that bristle color is less often affected by genetic agencies than either body color or color of the larval jaws. This may mean that less pigment-forming material is necessary for pigmentation of the bristles or, as is more likely, that bristle pigment is laid down at a time particularly favorable for melanin production.

Summary.—Pigmentation of the larval mouthparts and spiracle sheaths has been studied in the mutations of *D. melanogaster* which influence adult body or bristle color. Of the mutants characterized by darkened adult pigmentation, only the ebony alleles were found to increase pigmentation of the larval mouthparts. The ebony alleles considerably darken the spiracle sheaths at all stages of larval development; no other genetic factors were observed to have this effect. In general, the mutants whose adult body color is lighter than wild type are characterized by lighter pigmenta-

tion of the larval mouthparts. Bristle color appears to be affected less often than either body color or mouthpart pigmentation.

[1] Brehme, K. S., *Proc. Soc. Exp. Biol. Med.*, **37**, 578–580 (1937).
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[4] Sturtevant, A. H., *Pub. Carnegie Inst.*, **301**, 150 pp. (1921).
[5] Beadle, G. W., *Amer. Nat.*, **71**, 277–279 (1937).
[6] Graubard, M., *Jour. Genet.*, **27**, 199–218 (1933).

THE SPERMATOPHORES OF TRITURUS TOROSUS AND TRITURUS RIVULARIS

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During the course of studies on the breeding activities of the western newts[1] the writer has had occasion to observe the spermatophores of *Triturus torosus* (Rathke) and of the closely related *Triturus rivularis* (Twitty). Concerning the latter, Twitty[2] gives incidental reference to the deposition of a spermatophore by a captive male. However, since there is in the literature no other allusion to the spermatophore either of this species or of *T. torosus*,* the following observations appear pertinent.

It is known that the glands in the cloaca produce the secretions which form the spermatophores. Moreover, these glandular products may be traced by their staining reactions and composition from the glands of origin directly to the spermatophore. In *Triturus torosus* the pelvic glands in the roof of the cloaca produce a coarse granular secretion which is deeply eosinophilic in prepared material but opaque white in the fresh condition. Its function has been described for other species (cf. Noble,[3] p. 287, and Noble and Brady[4]) as being that of agglomerating the sperm cells into a mass, or sperm capsule, and subsequently of attaching this mass to a gelatinous supporting structure, the spermatophore stalk. Studies of the cloaca and sperm mass in *T. torosus* disclose that in this species also the secretions of the pelvic gland operate in holding the sperm together and in attaching the oblong mass to the apical horn of the stalk. This is further shown in the stained spermatophore, where the pelvic secretion may be seen covering the tip of the apical horn.

The arrangement of the sperm cells within the mass is of some interest and may be contrasted with that observed by Noble and Brady (loc. cit., p. 104) in *Ambystoma opacum*. Here it is stated: "The spermatozoa are spread over the top of the spermatophore. A remarkable feature is that

the heads of the spermatozoa are often all directed the same way. Thus in the micro-photograph (Fig. 90) of the head of one of the spermatophores it may be noticed that the spermatozoa covering one of the four summits are directed outward. This orientation of the spermatozoa would appear to be advantageous to a rapid impregnation." In *Triturus torosus* there is no marked orientation of the sperm, for they are variously agglomerated within the mass, usually in fascicles of perhaps 50 or more cells; the direction of any one of these fascicles appears to be largely independent of the others within the mass of the sperm capsule.

There is a single sperm capsule, or head, to the spermatophore of *Triturus torosus*. This is attached to the apex of the central member of the three recurving points (see Fig. 1) which crown the summit of the gelatinous stalk. The head retains its delicate attachment for only a short time after deposition, a very slight disturbance of the water being sufficient to dislodge it. Consequently, of the many spermatophores noted, both during and shortly after emission, but few were observed intact. None of these could be kept in this condition long enough to permit photographing.

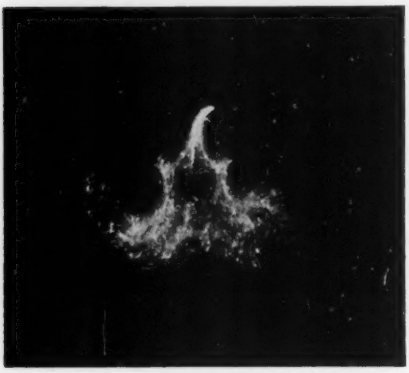

FIGURE 1

Frontal view of the spermatophore stalk of *Triturus torosus* (× 1.4). The opacity at the apex of the median projection marks the point of attachment of the sperm head to the stalk.

Measurements of the spermatophore parts, together with notes on gross appearance may be summarized as follows. *Head* (sperm capsule): shape, oblong when deposited, becoming spherical within ten minutes following deposition; length, 3.0 millimeters; width, 2.3 millimeters; coloration, opaque white; surface, irregular and poorly defined; substance, a viscous agglomeration of sperm throughout; restricted only by diffuse membrane; attachment, to apical horn of stalk by single terminal strand. *Stalk:* (Fig. 1) shape—apex consisting of three recurving points, the two laterals short, the median extended into a terminal falcate horn, with opaque filamentous axial portion; central portion of stalk roughly cylindrical, hollow and perforated laterally by an oval opening; base oblong in section, flaring abruptly from the central portion, with about twelve slightly opaque fibrillar lines radially arranged; substance, a firm gel, collapsing out of

water, resuming form when returned; coloration, transparent, colorless except for fibrillar opacity in central horn of apex; approximate measurements of stalk:

Height (base to apical falcate curve)	11.0 mm.
Width at base	16.0 mm.
Width at central portion	7.0 mm.
Width across two lateral prongs	8.0 mm.
Length of apical falcate horn from curve	1.5 mm.
Lateral width of falcate horn	0.8 mm.
Vertical width of falcate horn	
At curve	1.5 mm.
Tapering to	0.5 mm.
Lateral aperture (in central portion of stalk)	
Width	2.5 mm.
Height	7.0 mm.

The number of spermatophores deposited by individual males of *T. torosus* in the laboratory has never exceeded four, and the usual number under these conditions is two or one. On the assumption that the number of emissions will depend in general upon the amount of testicular material, considerable variation may be expected in the quantity of spermatophores deposited by individual males even in nature. This is contingent upon the fact that we are dealing with a somewhat heterogeneous age distribution in which the number of testicular lobes tends to increase as the age.[5]

While the spermatophores of *Triturus torosus* have been abundantly noted, their homolog in *T. rivularis* must be described from a single specimen obtained from one of three amplectic pairs confined in the laboratory aquaria. When first noted the head of the spermatophore had already become disengaged from its stalk, but both parts still remained in excellent condition; the latter circumstance indicated very recent deposition.

When compared with the spermatophore of *T. torosus* that of *T. rivularis* appeared to differ only in minor features. The stalk of the latter was similar but somewhat less rugged; there was less fibrillar scaffolding material at the apex, and the falcate horn appeared more delicate and mucid than in the *torosus* product. Measurements and photographs were not taken since the stalk was somewhat damaged during removal from the substratum. Because of the foregoing exigencies a more detailed comparison cannot be given at present. However, one further point of difference may be noted, namely, that the stalk of the spermatophore from *rivularis* was very firmly attached to the substratum.† By contrast, those of *torosus* have often remained fixed only momentarily and seldom for more than a few minutes.

Field data extend limited support to this apparent difference in degree of fixation; during the current breeding season the writer has obtained, for the first time in the field, spermatophore stalks of *torosus*. Two of

these were found, both floating near the surface in quiet water sinks some distance from the main breeding pond (located near Berkeley, California). Unfortunately, analogous data on *T. rivularis* are lacking, but it appears relevant to note certain differences in the typical breeding sites of the two species in question. *T. rivularis* ordinarily breeds in fairly rapid streams where a clean, rocky substrate prevails; *T. torosus*, on the other hand, prefers the quieter waters of ponds and eddy pools, situations which ordinarily afford little solid material as bases for spermatophore attachment. Thus, at least to the extent of the foregoing generalizations, it is possible that a stronger fixation of the stalk in *rivularis* would in some degree facilitate the mating of this species under the conditions of its breeding environs.

* Ritter (*Proc. Calif. Acad. Sci.*, ser. 3, Zool., **1**, 73–114, 1897) found certain masses of sperm from this species. Concerning them he states: "From my present knowledge these would be more properly called drops of semen than spermatophores." Ritter observed three such masses. One he found ". . . contained in and protruding from the cloaca of an amplectic female . . ." Another was found on the substratum of the aquarium in which was a captive male; a third was taken from the cloaca of a male. During the present work with *torosus* many such masses of sperm have been noted protruding from the cloaca of the female, and it is clear that these represent only the head of the spermatophore which the female ordinarily removes from the stalk after emission by the male.

† Dr. Twitty (oral communication) indicates that the spermatophore which he found deposited by *T. rivularis* in the laboratory was also firmly attached to the floor of the aquarium.

[1] Smith, R. E. (in press) (1941).
[2] Twitty, V. C., *Copeia, Ann Arbor*, **1935** (2), 73–78 (1935).
[3] Noble, G. K., *The Biology of the Amphibia*, McGraw-Hill (1931).
[4] Noble, G. K., and Brady, M. K., *Zoologica, New York*, **11** (8), 89–132 (1933).
[5] McCurdy, H. M., *Amer. Jour. Anat., Philadelphia*, **47**, 367–399 (1931).

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*ON A CLUSTER OF NEBULAE IN HYDRA**

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Clusters of nebulae are the largest known aggregations of matter which possess definite individual characteristics. In preparation of a theoretical study of the large scale distribution of matter in the universe we propose to analyze some simple structural features of a number of clusters of nebulae. Most of the observational data used were obtained with the 18-inch Schmidt telescope on Palomar Mountain. Photographs taken with this instrument, because of their excellent and uniform definition over a field of about

seventy square degrees are well suited for a survey of a number of the more near-by clusters of nebulae.

In the present report we shall be concerned with a cluster of nebulae in Hydra. The three nebulae NGC 3285, 3309 and 3312, the apparent magnitudes of which according to the Shapley-Ames catalog are $m = 13.2$, 12.7 and 13.1, respectively, lie near the line of sight of the center of the cluster

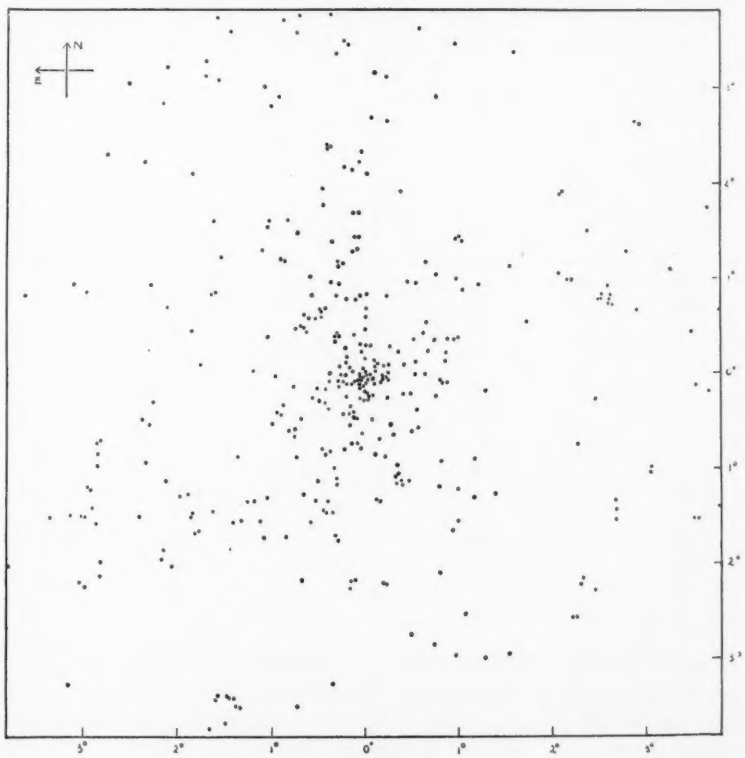

FIGURE 1
The hydra cluster of nebulae.

(at about R.A. $10^h\ 34^m$, Decl. $-27°\ 16'$, epoch 1950). The distribution of the nebulae brighter than about the apparent magnitude $m = 16$ is shown in figure 1.

In order to determine the radial distribution of the nebulae circles whose radii are equal to $n \times 10'$ were drawn around the center of the cluster and in each ring of $10'$ width the total number of nebulae was counted in

the four quadrants fixed by the north-south and the east-west directions. These counts are given in table 1. The number of nebulae in a ring of outer radius r is n_r.

TABLE 1

COUNTS OF NEBULAE IN RINGS WHOSE WIDTH IS 10 MINUTES OF ARC

NUMBER OF RING	QUADRANTS NE	SE	SW	NW	n_r	N_r
1	2	10	5	4	21	243.6
2	7	10	9	6	32	123.0
3	6	8	3	3	20	46.4
4	2	7	4	3	16	26.7
5	8	6	7	4	25	32.5
6	8	7	4	6	25	26.7
7	2	8	3	4	17	15.1
8	7	2	6	1	16	12.4
9	4	3	4	3	14	9.5
10	2	2	1	1	6	3.7
11	6	4	1	4	15	8.2
12	6	3	3	2	14	7.1
13	2	3	0	1	6	2.8
14	4	7	3	0	14	6.0
15	5	8	2	3	18	7.2
16	0	1	0	2	3	1.2
17	1	0	2	9	12	4.2
18	0	10	1	1	12	3.9
19	3	3	4	4	14	4.4
20	3	5	5	1	14	4.2
21	3	4	4	0	11	3.1
22	4	2	1	2	9	2.4
23	5	10	1	1	17	4.4
24	4	1	2	6	13	3.1
25	1	4	2	3	10	2.3
Total	95	128	77	74	374	

In all of the rings which together cover a circle of $25 \times 10' = 4° 10'$ of arc radius a total of 374 nebulae was counted. From table 1 it follows that the Hydra cluster, similar to the Coma cluster,[1] possesses great spherical symmetry and that it, therefore, seems to be a cluster which has very nearly reached a statistically stationary state. This conclusion is further borne out by the radial distribution of the nebulae. In column 7 the number N_r of nebulae per square degree in dependence of the distance r from the center is tabulated. In figure 2 the values of $\log_{10} N_r$ in dependence of r are plotted. The similarity of the radial distribution with that previously obtained for the Coma cluster[1] and with the distribution of matter to be expected for an Emden isothermal gravitational gas sphere is at once apparent. An intercomparison of different clusters and a discussion of the physical significance of the radial distribution curves will

be given in another place after some additional similar clusters have been analyzed.

According to Hubble[2] the absolute magnitude of the fifth brightest nebula in a cluster is about $M_5 = -16.4$. The apparent magnitude of the fifth brightest nebula in the Hydra cluster is about $m_5 = 13.2$. Introducing the usual correction for local obscuration[2] at the galactic latitude $\beta = 27°$ of the cluster the apparent magnitude m_5' of the fifth brightest nebula reduced to the galactic pole becomes

$$m_5' = 13.2 + 0.25\,(1 - \operatorname{cosec}\beta) = 12.9 \qquad (1)$$

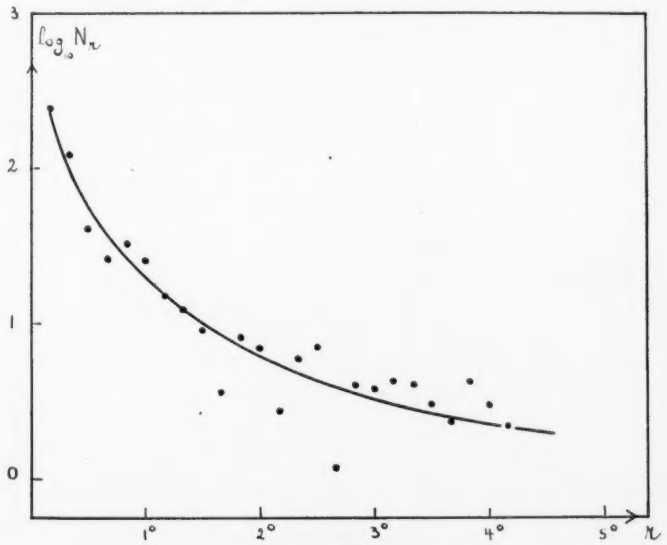

FIGURE 2

Radial distribution of nebulae in the hydra cluster.

and the distance p (in parsecs) of the Hydra cluster

$$\log_{10} p = 1 + (m_5' - M_5)/5 = 6.86 \qquad (2)$$

or

$$p = 7.3 \times 10^6 \text{ parsecs} = 24 \text{ million light years} \qquad (3)$$

The red shifts (apparent velocities v of recession) for nebulae in the Hydra

cluster according to Hubble should be expected to be given by the relation

$$\log_{10} v = 0.2\, m_5' + 1.025 = 3.615 \tag{4}$$

or

$$v \cong 4100 km/\text{sec} \tag{5}$$

From the curve in figure 2 it follows that the Hydra cluster has a diameter of at least ten degrees and that the number N_β of nebulae per square degree in the surrounding field is of the order $N_\beta = 1.8$ ($\log_{10} N_\beta = 0.25$). Correcting for local obscuration and for zenith distance[3] the number of nebulae which should be expected if the Hydra cluster were located at the galactic pole and if it could be observed at the zenith distance zero, is

$$\log N = \log N_\beta - 0.15(1 - \operatorname{cosec} \beta) + \Delta Z \tag{6}$$

where according to Hubble[3] for a zenith distance of $Z = 63°$ we have $\Delta Z \cong 0.20$ and therefore

$$\log N = 0.615 \tag{7}$$

Since for nebular counts including all nebulae brighter than the limiting apparent magnitude m_L it is[3]

$$\log N = 0.6 m_L - 9.1$$

our counts of the Hydra cluster roughly include all nebulae whose magnitude corrected for local obscuration is of the order

$$m_L = 16.2 \tag{8}$$

In the previously given analysis[1] of the Coma cluster of nebulae which lies near the northern galactic pole and which consequently at Palomar can be photographed near the zenith, the corresponding limiting magnitude at which nebulae could safely be distinguished from stars on photographs obtained with the 18-inch Schmidt telescope was

$$m_L = 16.6 \tag{9}$$

The Hydra cluster cannot at Palomar be photographed at any zenith distance smaller than 63°, for which an extinction $\Delta m_p = 0.30$ in apparent photographic magnitude must be expected, a fact which satisfactorily accounts for the difference between the values (8) and (9).

The roughly 200 nebulae in the Hydra cluster counted in this investigation cover a range of only three magnitudes and therefore presumably represent less than half the population of this cluster whose further analysis with

the help of larger wide angle telescopes such as the 48-inch Schmidt telescope now under construction should prove profitable.

* Fifty-six nebulae covering 0.43 square degrees near the center of the Hydra cluster are included as group 5 in Shapley's list of groups of nebulae (*Proc. Nat. Acad. Sci.*, **19**, 591 (1933).

[1] Zwicky, F., *Astrophys. Jour.*, **86**, 217 (1937).

[2] Hubble, E., *The Realm of Nebulae*, pages 71, 169, etc., Yale University Press (1936).

[3] Hubble, E., *Astrophys. Jour.*, **79**, 139 (1934).

ENZYMES IN ONTOGENESIS. XVI. ACTIVATION OF PROTYROSINASE BY SODIUM ALKYL SULFATES[*,1]

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Communicated April 5, 1941

A number of compounds—sodium oleate, sodium taurocholate, Aerosol and Duponol—which activate protyrosinase contain both polar and non-polar groups (Bodine and Allen,[2] Bodine and Carlson[5]). Thus the property of amphipathy is common to these activators.† Since this property appears to be fundamental for an explanation of their behavior in solution, it ought to be useful to test the members of a homologous series in which the polar group is kept constant and the non-polar group is varied. This paper, therefore, describes the activation of protyrosinase by sodium alkyl sulfates with from eight to eighteen carbon atoms in the non-polar chain.‡

Diapause grasshopper (*Melanoplus differentialis*) eggs to the mass of 41.7 g. were triturated with mortar and pestle, diluted with 0.9 per cent NaCl, and centrifuged. The lipoidal layer was aspirated off and the supernatant decanted onto 0.2 g. of KH_2PO_4. After two hours at room temperature, this fluid was centrifuged. The resulting supernatant was made to 10 per cent with $(NH_4)_2SO_4$. An hour later the precipitate was centrifuged away and the supernatant made to 25 per cent $(NH_4)_2SO_4$. After a similar interval the precipitate was centrifuged down, mixed with 100 cc. of 0.9 per cent NaCl, and placed at 0°C. within a cellophane dialyzing tube. This tube floated in a liter of 0.9 per cent NaCl solution, which was thrice renewed within a four-day period. Next, the fluid from the tube was centrifuged and the clear, straw-colored supernatant was stored at 0°C. This extract, which was used throughout the course of the following experiments, contained a quantity of protyrosinase but no tyrosinase (Bodine and Allen[4]) and in itself showed no oxygen uptake.

The Warburg apparatus was used to determine the rate of oxygen uptake at 24.9°C. for the enzymic oxidation of tyramine to melanin. A

typical experimental arrangement had 0.3 cc. of the protyrosinase extract, 1.0 cc. of 0.1*M* Soerensen's phosphate buffer solution of pH equal to 6.8, a varying volume of a sodium alkyl sulfate solution in 0.9 per cent NaCl, and a compensating volume of 0.9 per cent NaCl solution in the reaction chamber of Warburg-Erlenmeyer type flasks. The side bulbs carried 0.3 cc. of 0.4 per cent tyramine HCl, the substrate, which was decanted into the reaction chambers one hour (Fig. 1) after the mixing of the other reagents. In all instances the fluid volume, or reaction volume, was equal to 3.0 cc.

Optically clear stock solutions of the alkyl sulfates in the presence of electrolytes were obtained by employing the following procedures. The sodium octyl, decyl and dodecyl sulfates were in 0.9 per cent NaCl at room

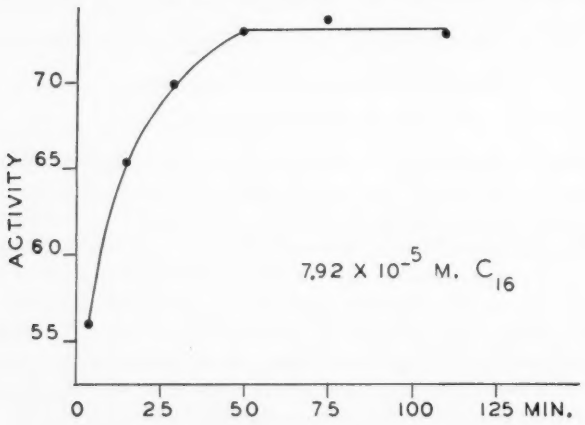

FIGURE 1

The attainment of equilibrium during the activation of protyrosinase by sodium hexadecyl sulfate.

temperature. The sodium tetradecyl and hexadecyl sulfates were in 0.9 per cent NaCl at 35°C. Since the sodium octadecyl sulfate was in redistilled water at room temperature, a more concentrated NaCl solution was added to the Warburg flask so that the electrolyte concentration would be equal to that for the other alkyl sulfates.

Tyrosinase activity (Fig. 2) was recorded by a graphic method of interpolation for the reciprocal of the time in minutes necessary for the initial 100 c. mm. of oxygen uptake. For convenience of expression this value is multiplied one thousand times.

The ascending limbs of these six concentration functions (Fig. 3) appear to show the increase in tyrosinase and the decrease in protyrosinase that occur in the presence of sodium alkyl sulfates. In the absence of an alkyl

sulfate there is no perceptible tyrosinase activity. Consequently, the enzyme, being inactive, is considered to be entirely present as a protyrosinase. But tyrosinase activity increases with increasing amounts of these sodium alkyl sulfates until with an excess of activator the enzymic oxidation of tyramine utilizes 100 c. mm. of oxygen in 11.1 to 11.2 minutes. Prior to the regions through which the protyrosinase to tyrosinase reaction

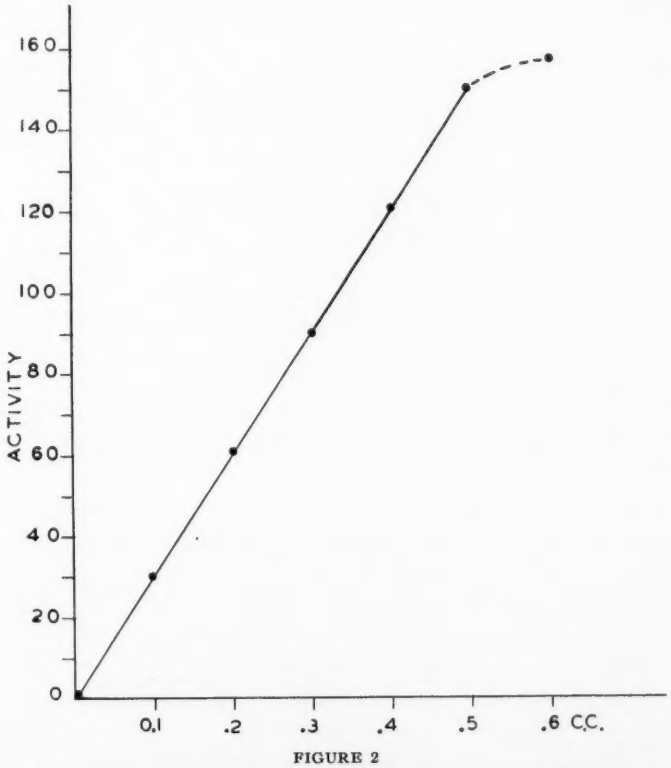

FIGURE 2

The tyrosinase activity of various amounts of the protyrosinase extract in the presence of excess sodium tetradecyl sulfate.

approaches completion, there is a part in the activation functions of the lower members of the series where changes in slope occur. These changes become less marked as the series is ascended and disappear with sodium hexadecyl sulfate. Since discontinuities are a feature of colloidal systems (von Buzágh[6]), the occurrence of this particular discontinuity was deter-

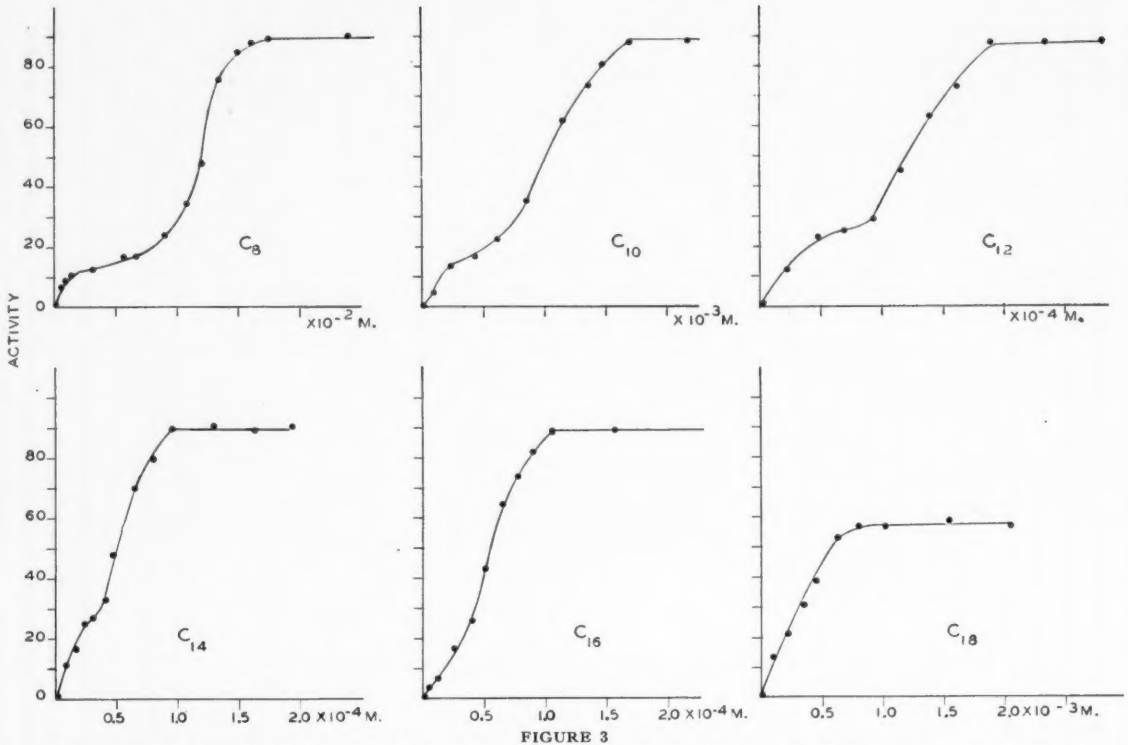

FIGURE 3

The activation of protyrosinase by various alkyl sulfates with the indicated number of carbon atoms in the alkyl group.

mined from the second derivative curve plotted with the aid of a tangentmeter. Minima corresponding to the "kinks" in the activation functions were thus obtained. The latter change is at $0.0035M$ for sodium octyl sulfate, $0.00035M$ for the decyl sulfate, $0.000065M$ for dodecyl sulfate

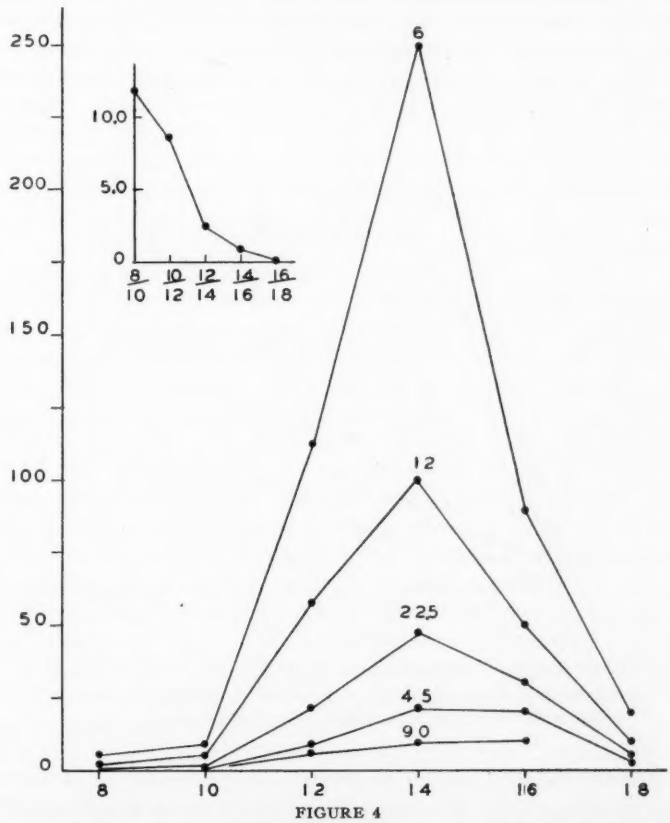

FIGURE 4

The efficiency of various sodium alkyl sulfates in the activation of protyrosinase. Abscissa, number of carbon atoms in the alkyl group; ordinate, reciprocal of the concentration times 10^3 for the indicated tyrosinase activities. The ratios of the concentrations which produce half activation are illustrated by the inset.

and $0.000030M$ for tetradecyl. Although no explanation for this phenomenon is offered by the authors, it seems worth noting that the transition in form resembles an S-shaped adsorption isotherm in contrast to that of the Langmuir type (Emmett[7]).

The activation isotherms appear to vary with the constitution of the activator molecules. The efficiency of the activators or the reciprocal of the concentration for a certain degree of activity is expressed in figure 4. With increasing number of carbon atoms, through eight to fourteen, there is a decrease in the number of molecules necessary for a certain degree of activity. Beyond fourteen carbon atoms increasing amounts of alkyl sulfates are needed. Presumably, the latter shift can be attributed to the insolubility of the octadecyl sulfate. A precipitate forms in dispersions of the latter compound in this electrolyte solution at 25°C. Thus, it is

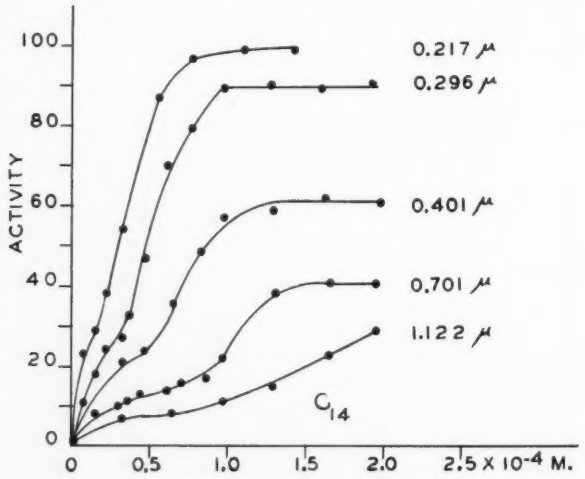

FIGURE 5

The effect of the ionic strength of the dispersion medium on the activation of protyrosinase by sodium tetradecyl sulfate. In this instance the activator is probably being "salted out."

probable that the low value for the ultimate tyrosinase activity with sodium octadecyl sulfate (Fig. 3) is due to a constraint which is introduced not through lack of protyrosinase but rather through lack of activator. Increasing the ionic strength of the reaction medium, aside from showing a general salt effect on tyrosinase (Allen and Bodine[1]), seems to cause an increase in the concentration of sodium hexadecyl sulfate needed for a certain degree of activity (Fig. 5). A visible correlate of the latter shift is the "salting out" of sodium hexadecyl sulfate. The real efficiency of the alkyl sulfates appears, therefore, to be complicated by the introduction of a solubility factor that probably does not operate among the lower members of the series. Solubility, a technical problem, and amphipathy, a funda-

mental property, seem to account for the form of the activation function and accordingly for the efficiency.

Sodium alkyl sulfates belong to a class of compounds which are believed to be dispersed in solution according to an equilibrium existing between simple ions and micelles composed of them (Hartley[9]). The constitution of the molecule, the total concentration, the temperature and the ionic composition of the dispersion medium subject this equilibrium to abrupt shifts in the favor of micelles. Thus, for example, the addition of ordinary electrolytes affects the adherent "gegenions" and consequently depresses the critical concentration for the formation of micelles (Hartley[9]). Since protyrosinase is unstable toward various reagents and treatments and is liable to change abruptly into tyrosinase (Bodine and Allen[3]), the activation isotherms of protyrosinase ought to be compared with the formation of micelles. A prime question has then to do with the total concentration at which micelles of sodium alkyl sulfates begin to form.

The quantity of an alkyl sulfate in the tyrosinase reaction medium can be increased so that its concentration is greater than would be the critical concentration for micelles in aqueous solution. After such a concentration is attained or surpassed, the tyrosinase remains at the constant, high degree of activity. Since Hartley found micelles of sodium cetyl sulfonate in decinormal acetic acid-acetate buffer solutions (Hartley[8]) at so low a concentration as $0.0001M$, there seems to be some circumstantial evidence for the occurrence of micelles through those regions where the protyrosinase to tyrosinase reaction is dependent on the concentration of activator. In water at 60° the critical concentration for micelles is $0.0076N$ for sodium dodecyl sulfate, $0.0028N$ for sodium tetradecyl sulfate, $0.00109N$ for sodium hexadecyl sulfate and $0.00039N$ for sodium octadecyl sulfate. These values bear a ratio of 2.7, 2.6 and 2.8 to each succeeding member of the series (Hartley[10]). The inset of figure 5 shows a similar comparison, but the ratio, 11.9 to 0.1, is not constant for the concentration of activator which produces half activation.

This comparison apparently leads to the conclusion that if micelles are involved in the activation of protyrosinase, they appear at concentrations not uniformly related to those where micelles form in aqueous solution. Moreover, the least concentration for full tyrosinase activity is lower, respectively, by some 40, 29 and 10 times that concentration for occurrence of micelles of dodecyl, tetradecyl and hexadecyl sulfates in water. Because the concentration for micelles is probably depressed to a relatively greater extent, it seems then that the effect of the reaction medium is of a greater magnitude with the short chain than with the long chain compounds.

Summary.—A protyrosinase from eggs of a grasshopper, *Melanoplus differentialis* (Thomas), can be activated by various alkyl sulfate compounds with from eight to eighteen carbon atoms in the alkyl group. This

protyrosinase seems to change into tyrosinase under the same conditions that favor the aggregation of the long chain ions into micelles composed of these ions. The latter conditions are discussed in terms of the prevailing concentration of ordinary electrolytes in the protyrosinase-tyrosinase reaction medium. It is hoped that this study will lead to the future elucidation of the mechanism for the activation of protyrosinase.

* Aided by a grant from the Rockefeller Foundation for research in cellular physiology.

† G. S. Hartley[9] writes, p. 44, "The property is essentially the simultaneous presence of separately satisfiable *sym*pathy and *anti*pathy for water. I propose, therefore, to call this property '*amphi*pathy'—the possession of both feelings."

‡ These six sodium alkyl sulfate compounds were a gift from E. I. du Pont de Nemours and Company to whom the authors are very grateful. The compounds were prepared for research purposes from alcohols of more than 99 per cent purity.

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FIELD PROPERTIES OF THE DEVELOPING FROG'S EGG

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Examination of the biological literature reveals that all living things, wherever they have been studied, exhibit electrical phenomena. They are present in two forms. The first of these is the alternating current phenomena recorded, for example, in the electrocardiograph and the electroencephalograph. The second is the relatively steady state direct current phenomena found in association with resting organisms and during activity and injury. The widespread occurrence of such electrical properties and their usefulness in the analysis of heart and brain activity attest their importance.

The majority of the studies of the electrical properties of living things,

however, have been isolated records of specific kinds of electrical phenomena. In other words, there has been no basic understanding of the origin of the electrical energy nor its exact relationship to fundamental biological activity. As a step toward clarifying and unifying the great body of evidence which has already been collected, Burr (1932) and Burr and Northrop (1935) proposed that the living organism be viewed as the morphological expression of an electrodynamic field. Briefly stated, this theory holds that in all living mechanisms cell membranes and phase boundaries set up potential gradients in protoplasm and that the voltage gradients so created determine in the living system an electrodynamic field. The field, established in this manner, controls and regulates the physical chemical processes by means of which the morphological pattern is established. The field forces, measured in electrical terms, then become the determiners of the pattern of organization of the organism. The concept of field in development is by no manner of means new, for it has been well established in the techniques of experimental embryology. The electrodynamic field theory attempts to add quantitative electrical definition of the field, thereby substituting reasonably exact measurement for qualitative description. If this theory can be demonstrated, a simple basic explanation of both the AC and the DC electrical properties of organisms can be given. The relatively steady state DC potentials define the field, and the AC phenomena can then be understood as local variation in the field produced by the characteristic activity of a specific organ such as the heart or the brain. The theory follows logically enough from evidence in the literature but for complete satisfaction the theory must be validated by experimental study.

In order to test the correctness of the concept, four questions require answers:

1. Do living things possess relatively steady state potential differences which can be measured with reasonable certainty?
2. Are the voltage gradients so measured found in some recognizable pattern?
3. Do these voltage gradients define an electrodynamic field?
4. Since the field exists, is it a kind of electrical photograph of biological activity, or can it be in any sense of the word a determiner of the pattern of organization?

For the past six years, evidence has been slowly accumulating which shows that the answers to the first three questions must be affirmative (Burr and Northrop, 1939). The fourth question is yet to be settled.

Obviously, if the field determines the pattern of organization, it should be possible to change development of an organism by modifying the field. However, a little calculation has shown that the field intensities in cellular units of the living system are so great that the construction of an external

DC field, sufficiently large to modify them, is either physically impossible or prohibitively expensive. Modification of the field, however, by an external AC field has been accomplished, for it is well known that bombardment of an organism in the early phases of development by x-rays produces definite developmental deficiencies.

It is clear, then, that the proof of the importance of the DC field must be derived from indirect evidence. If, for example, the unfertilized or undivided egg of such a form as a frog possesses an electrical pattern which can be traced through in a significant relationship to the morphological

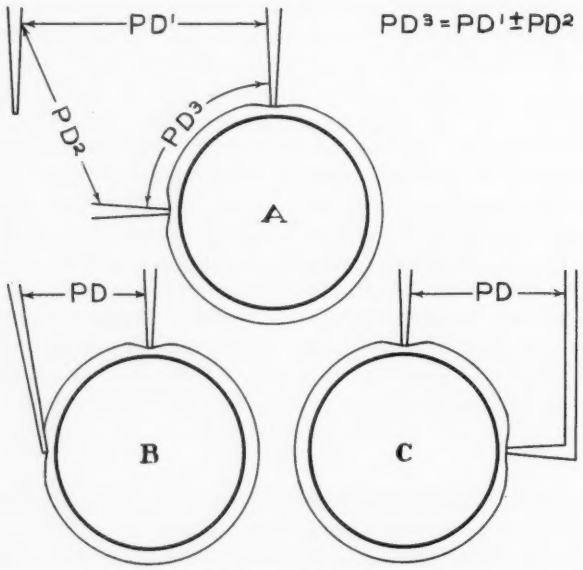

FIGURE 1

Diagram of three methods of electrode application.

pattern, the implication would be very great that the field defined by the electrical pattern determines the form of the ensuing embryo.

The present report is a study of some six thousand determinations on fifty frogs' eggs prior to the development of the primary axis of the embryo as seen in the appearance of the medullary plate. Potential differences between the vertex of the animal pole and four equidistant points on the equator of the egg were measured by the Burr-Lane-Nims (1936) technique. Three variations in the technique were employed (Fig. 1) and all were subjected to statistical analysis.

In the early experiments, one micro tip leading to the salt bridge of a silver-silver chloride electrode was brought vertically downward on to the gelatinous membrane which covers the egg. Pressure was exerted by the micromanipulator just sufficient to deform the outer surface of this layer. This meant, of course, that the tip itself was separated from the egg by the vitelline membrane and a fraction of a millimeter of jelly. The second electrode was brought downward to the equator of the egg and, by the manipulator, moved medially until it likewise indented the jelly. It will be

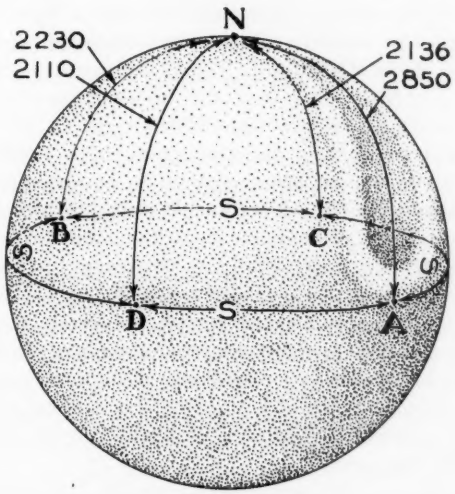

FIGURE 2

Diagram of voltage gradients in microvolts between *N*, the vertex of the animal pole, and four points on the equator of the egg showing their relationship to the primary axis of the nervous system. *N* is negative to the points on the equator and to the solution. *S* indicates statistically significant differences between equatorial points.

noted that the geometry of the contact surfaces of the electrode tips differed. The first electrode at the vertex of the animal pole presented a circular opening approximately 100 μ in diameter whose plane was tangent to the surface. Electrode two at the equator presented a similar opening, the plane of which was at right angles to the surface. Under these conditions the voltage differences tended to be relatively low. The first variation of the technique was made by bringing the equatorial electrode into contact with the surface so that the plane of the opening of the micro tip was

tangent to the surface as was the opening of the vertical electrode. Under these conditions the same pattern was determined but the magnitude of the voltage gradients was greater. As a final check on the procedure, one electrode was used as a reference electrode a centimeter at least away from the egg in the surrounding solution. The exploring electrode was then brought to the vertex in a tangent relationship and then successively to the four points on the equator in the same tangent relationships. Here again the pattern was detected with voltage gradients approximately equal to the first technical variant.

From the very beginning it was apparent that at least one of the four points on the equator showed a significantly greater voltage drop from the vertex than any of the other three. This point was then marked either by staining with Nile Blue sulphate or by the establishment of orientation points in the immediate environment of the egg. Of the fifty embryos, ten were marked successfully and developed to the neural plate stage. In the remaining forty, correlations were either not attempted or were impossible because of technical difficulties. It should be noted that the technique is rigorous and requires great care in its manipulation, and technical artifacts, therefore, frequently creep into the study. In every case where correlations were possible, the primary axis of the organism came to lie in the plane of the greatest voltage drop from the vertex (Fig. 2). In other words, it was possible to predict from the voltage pattern where the head of the organism would develop. It is hard to escape the conclusion, therefore, that the electrical pattern is primary and in some measure at least determines the morphological pattern. Added weight is given to the argument by the following facts.

Typical field patterns have been observed in the unfertilized egg during three or four days subsequent to laying. When obvious signs of cell death appeared, the voltage gradients disappeared. In like manner, the same pattern was to be seen in the fertilized but undivided egg. Moreover, it can be followed through the early stages of segmentation, the formation of the morula, the gastrula, and even shows the same characteristics in the early medullary plate stage. It seems clear from the above that, at least in the frog's egg (and in the egg of Amblystoma, for some preliminary studies in the latter confirm these findings), there exists an electrical field which appears prior to the appearance of the formed structures and also predicts the longitudinal axis of the future embryo.

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INVARIANT FORMS OF INTERACTION BETWEEN NUCLEAR PARTICLES

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1. The theories of nuclear forces are as yet so uncertain that it is not possible to draw definite conclusions concerning the wave equation between the heavy particles in nuclei. As has been observed repeatedly, it is even questionable whether one can achieve reasonable accuracy by using a wave function which contains only the coördinates of the heavy particles as variables. It is quite conceivable that no description with such a wave function is adequate. It is possible that one should use, even for the description of the stationary states, a wave function which contains, in addition to the coördinates of the protons and neutrons, the coördinates of the light particles (e.g., mesons) which transmit the interaction. The question is similar to that arising in the theory of molecules, and, in particular, chemical reactions, where one can use a Hamiltonian for the heavy particles alone only if the adiabatic hypothesis applies, i.e., if the motion of the light particles is much more rapid than that of the heavy particles. In this case the former, considered alone, are in the lowest state for every configuration of the heavy particles. The usual condition for this is*

$$2\pi h\nu \gg (dE/dt)\ (1/\nu) \approx (dE/dx)(v/\nu) \qquad (1)$$

where $2\pi h\nu$ is the energy necessary for the excitation of the light particles, dE/dt the change of interaction energy between the heavy particles in unit time, v the velocity of the heavy particles. The right side of (1) represents the change in interaction energy during one period in the motion of the light particles. Inserting twice the rest energy, μc^2, of the meson for $2\pi h\nu$ a velocity v corresponding to a mean kinetic energy of $\mu c^2/_3$, and a dE/dx, which is equal to $\mu c^2/(2\pi h/\mu c)$, the left side becomes about 15 times greater than the right. This shows that it is reasonable to look for a wave equation for the heavy particles alone. However, one may do well to keep an open mind on this question because of the crudeness of the above estimates. Thus, e.g., it is quite possible that the excitation potential of the meson

field in the presence of heavy particles is a good deal smaller than the excitation energy $2\mu c^2$ in free space.

2. We shall, in the following, assume the existence of a Hamiltonian for the heavy particles alone and investigate the possible types of interaction. We shall further assume that the interaction is between pairs of particles,[1]

$$V = {}^1/_2 \sum_{i \neq k} V_{ik} \tag{2}$$

It will be supposed that we can restrict ourselves to a non-relativistic approximation. It has been shown by Breit[2] that a relativistic treatment would necessitate the addition of rather complicated correction terms to all the primitive interactions to be enumerated. Furthermore, we shall restrict ourselves to interactions which either do not contain the velocities (or momenta) of the heavy particles or contain them only to the first power.

For the description of the heavy particles we shall use the formalism of the isotopic spin.[3] According to Cassen and Condon,[4] this permits a simpler description of some types of interaction than the other formalisms. It permits us to assume that the wave function is antisymmetric with respect to permutations involving space, ordinary spin and isotopic spin coördinates. The condition for antisymmetry is

$$P_{ik}Q_{ik}T_{ik}\Psi = -\Psi \tag{3}$$

where P_{ik} is the operator which exchanges the space coördinates of particles i and k, and $Q_{ik} = {}^1/_2 + {}^1/_2(\mathbf{s}_i, \mathbf{s}_k)$ and $T_{ik} = {}^1/_2 + {}^1/_2(\tau_{i\xi}\tau_{k\xi} + \tau_{i\eta}\tau_{k\eta} + \tau_{i\zeta}\tau_{k\zeta}) = {}^1/_2 + {}^1/_2(\tau_i \cdot \tau_k)$ exchange the ordinary spin and isotopic spin coördinates, respectively. The identity (3) makes it possible to give different forms to any given operator. In particular, the operator of the exchange of space coördinates, for which the expressions in a formalism not using the isotopic spin are rather complicated,[5] can be written as[4]

$$P_{ik} = -Q_{ik}T_{ik} = -{}^1/_4(1 + (\mathbf{s}_i \cdot \mathbf{s}_k))(1 + (\tau_i \cdot \tau_k)) \tag{3a}$$

We shall use eight conditions to limit the possible forms of interaction. These are that the interaction operators V_{ik} must

(1) depend only on differences of space coördinates (invariance under displacements);

(2) be hermitean (independent of the choice of the beginning of the time scale);

(3) have spherical symmetry (invariant under rotations);

(4) depend only on the difference of momenta (this will be shown to follow from the invariance under Galilei transformations);

(5) be invariant with respect to inversion;

(6) be invariant with respect to the substitution of $-t$ for t (time reversal);

(7) be symmetric in the particles;

(8) be consistent with the law of conservation of electric charge.

Only the fourth point needs further elucidation. In the non-relativistic theory, with which we are working, the wave function of a state $\Psi(x_1, x_2, \ldots, x_n, t)$ is, in a coördinate system moving with the velocity v in the x direction,

$$\bar{\Psi}(x_1, \ldots x_n, t) = \Psi(x_1 - vt, \ldots x_n - vt) \cdot e^{i(m_1x_1 + \cdots + m_nx_n)v} \cdot e^{-i(m_1 + \cdots + m_n)v^2t/2h} \quad (4)$$

The infinitesimal operator corresponding to this transformation is

$$-t\left(\frac{\partial}{\partial x_1} + \frac{\partial}{\partial x_2} + \cdots + \frac{\partial}{\partial x_n}\right) + i(m_1x_1 + \ldots + m_nx_n) \quad (4a)$$

We can consider an arbitrary operator as a function of the $\mathbf{s}$, the τ, the coördinates of the center of mass $m_1\mathbf{x}_1 + \cdots + m_n\mathbf{x}_n$, the differences between coördinates, $x_1 - x_2$, $y_1 - y_2$, $z_1 - z_2$, $\cdots$, the total momentum $-ih(\partial/\partial x_1 + \cdots + \partial/\partial x_n)$ and the differences between the velocities $-ih(m_1^{-1}\partial/\partial x_1 - m_2^{-1}\partial/\partial x_2)$. If expressed in terms of these quantities, the interaction operator certainly cannot depend on the coördinates of the center of mass because it must be invariant with respect to displacement. The condition that it be invariant under Galilei transformations requires that it commute with (4a). All of the above quantities—and thus all functions of them —commute with (4a) except for the coördinate of the center of mass which cannot enter into the interaction anyway, and except for the total momentum in the x direction, $-ih(\partial/\partial x_1 + \cdots + \partial/\partial x_n)$. Hence the interaction operator must not depend on this momentum. For similar reasons, it must be independent of $-ih(\partial/\partial y_1 + \cdots + \partial/\partial y_n)$ and $-ih(\partial/\partial z_1 + \cdots + \partial/\partial z_n)$. It can depend, however, on the relative velocities for which the differences of momenta can be substituted because of the approximate equality of the masses. This justifies point 4 above.

Because of 7 and our assumption of pair interactions, it is sufficient to find the most general interaction for a single pair of particles, say the particles 1 and 2.

A discussion similar to that which follows has been carried out before.[6] However, because of an oversight, one type of interaction was given as permissible although it should have been excluded on the basis of the above eight conditions. The present paper contains, furthermore, some relations between the different types of interactions which may prove useful in further work.

3. We shall proceed now as in reference 6 to determine those functions of the components of the vectors $\mathbf{s}_1$, $\mathbf{s}_2$, $\mathbf{r} = \mathbf{x}_1 - \mathbf{x}_2$, $\mathbf{p} = \mathbf{p}_1 - \mathbf{p}_2$ and of

$\tau_{\xi 1}$, $\tau_{\eta 1}$, $\tau_{\zeta 1}$, $\tau_{\xi 2}$, $\tau_{\eta 2}$, $\tau_{\zeta 2}$ which satisfy our eight invariance conditions. We can restrict ourselves to linear functions of the $\mathbf{s}$ and τ (these quantities have no functions of higher degree) and also of $\mathbf{p}$, as we have decided to disregard higher order terms in this variable.

In this section we shall investigate only functions which do not depend on the τ; the dependence on τ will be introduced in section 4.

There are 16 different functions of the spin variables. These are two scalars

$$1 = 1; \quad Q_{12} = {}^1/_2 + {}^1/_2(\mathbf{s}_1 \cdot \mathbf{s}_2), \tag{5a}$$

the nine components of three axial vectors

$$\left.\begin{aligned} w_x^{(1)} = s_{x1} + s_{x2}, \cdots; \quad w_x^{(2)} = s_{x1} - s_{x2}, \ldots \\ w_x^{(3)} = s_{y1}s_{z2} - s_{z1}s_{y2}, \ldots, \end{aligned}\right\} \tag{5b}$$

and the five independent components of an ordinary symmetric tensor with trace zero

$$t_2 = {}^1/_2(s_{x1} + is_{y1})(s_{x2} + is_{y2}); \ldots. \tag{5c}$$

We shall obtain all functions of the spin and momentum coördinates in which we are interested by combining the above functions of the $\mathbf{s}$ with 1 and the vector $\mathbf{p} = \mathbf{p}_1 - \mathbf{p}_2$. In this way, (5*a*) gives two scalars, 1 and Q_{12}, and two ordinary vectors, $\mathbf{p}$ and $Q_{12}\mathbf{p}$; (5*b*) gives three axial vectors $\mathbf{w}^{(1)}$, $\mathbf{w}^{(2)}$, $\mathbf{w}^{(3)}$; three scalars $(\mathbf{w}^{(1)} \cdot \mathbf{p})$, $(\mathbf{w}^{(2)} \cdot \mathbf{p})$, $(\mathbf{w}^{(3)} \cdot \mathbf{p})$; three ordinary vectors $[\mathbf{w}^{(1)} \times \mathbf{p}]$, $[\mathbf{w}^{(2)} \times \mathbf{p}]$, $[\mathbf{w}^{(3)} \times \mathbf{p}]$ and three symmetric axial tensors with zero trace which have components like $t_2^{(1)} = {}^1/_2(w_x^{(1)} + iw_y^{(1)}) \cdot (p_x + ip_y)$; (5*c*) gives in addition to the tensor (5*c*), one ordinary vector which can be written as $\mathbf{w} = \mathbf{s}_1(\mathbf{s}_2 \cdot \mathbf{p}) + \mathbf{s}_2(\mathbf{s}_1 \cdot \mathbf{p})$, a symmetrical axial tensor with zero trace, the components of which have the form

$$T_2 = (s_{1x} + is_{1y}) \cdot ([\mathbf{s}_2 \times \mathbf{p}]_x + i[\mathbf{s}_2 \times \mathbf{p}]_y) + (s_{2x} + is_{2y})([\mathbf{s}_1 \times \mathbf{p}]_x + i[\mathbf{s}_2 \times \mathbf{p}]_y),$$

and, finally, a tensor of the third rank with seven independent components.

Thus, we have five scalars, nine vectors, five symmetric tensors with zero trace and one tensor of the third rank, with 64 components altogether. These must be combined with all possible functions of the distances $x = x_1 - x_2$, $y = y_1 - y_2$, $z = z_1 - z_2$, ... and the τ. Since the τ naturally are invariant with respect to rotations, we shall have to form scalars out of the above quantities and the distance vector $\mathbf{r}$.

It is well known that the most general scalar which can be formed from the components of a vector $\mathbf{r}$ is an arbitrary function of the length r of the vector. Similarly the components of the most general vector, and sym-

metric tensor with trace zero have the form $xf(r)$, $yf(r)$, $zf(r)$ and $(x + iy)^2 \cdot g(r)$, etc., respectively, where again f and g are arbitrary functions of r. The tensors of higher ranks have similar forms. The scalars obtained above are ordinary scalars, i.e., they do not change sign if one goes over from a right-handed to a left-handed coördinate system, and this holds for all the above tensors of even rank. The vectors, on the other hand, are polar and this holds for all tensors of odd rank.

In order to satisfy condition 3, we must combine the scalar functions of $\mathbf{s}_1$, $\mathbf{s}_2$, $\mathbf{p}$, with scalar functions of $\mathbf{r}$, the vector functions with vector functions, etc. Interactions which differ only in having a different dependence on r can be said to be of the same *type*. Hence only twenty types of interaction exist (corresponding to the 5 scalars, 9 vectors, 5 tensors of second and 1 of the third rank) which satisfy conditions 1, 4 and 3. If we add to these condition 5, we must remember that $\mathbf{s}_1$ and $\mathbf{s}_2$ are axial while $\mathbf{p}$ is a polar vector. Hence, we must exclude the three scalars obtained from (5*b*) because they are pseudoscalars and remain pseudoscalars if multiplied by a function of r. Similarly, we must exclude the vectors $\mathbf{w}^{(1)}$, $\mathbf{w}^{(2)}$, $\mathbf{w}^{(3)}$, also obtained under (5*b*) because they are axial, and the tensor $\mathbf{t}^{(1)}$ under (5*b*) because it changes sign in the transition from a right-handed to a left-handed coördinate system. From the forms obtained under (5*c*) the tensor of the second rank, $\mathbf{T}$, must be excluded. Only two scalars, six vectors, one tensor of the second and one of the third rank remain to give real scalars with functions of the components of $\mathbf{r}$. Disregarding the arbitrary factor which is a function of r, the remaining 10 types can be written in the following forms:

$$1; \qquad Q_{12}; \tag{6a}$$

$$(\mathbf{p}\cdot\mathbf{r});\ Q_{12}(\mathbf{p}\cdot\mathbf{r});\ (\mathbf{r}\cdot[\mathbf{w}^{(1)}\times\mathbf{p}]);\ (\mathbf{r}\cdot[\mathbf{w}^{(2)}\times\mathbf{p}]);\ (\mathbf{r}\cdot[\mathbf{w}^{(3)}\times\mathbf{p}]); \tag{6b}$$

$$\left.\begin{aligned}&(\mathbf{r}\cdot\mathbf{s}_1)(\mathbf{s}_2\cdot\mathbf{p}) + (\mathbf{r}\cdot\mathbf{s}_2)(\mathbf{s}_1\cdot\mathbf{p}) - {}^2/_3(\mathbf{r}\cdot\mathbf{p})(\mathbf{s}_1\cdot\mathbf{s}_2);\\ &(\mathbf{r}\cdot\mathbf{s}_1)(\mathbf{r}\cdot\mathbf{s}_2) - {}^1/_3r^2(\mathbf{s}_1\cdot\mathbf{s}_2);\end{aligned}\right\} \tag{6c}$$

$$(\mathbf{r}\cdot\mathbf{s}_1)(\mathbf{r}\cdot\mathbf{s}_2)(\mathbf{r}\cdot\mathbf{p}) - {}^1/_5r^2\{(\mathbf{s}_1\cdot\mathbf{s}_2)(\mathbf{r}\cdot\mathbf{p}) + (\mathbf{s}_1\cdot\mathbf{p})(\mathbf{r}\cdot\mathbf{s}_2) + (\mathbf{s}_2\cdot\mathbf{p})(\mathbf{r}\cdot\mathbf{s}_1)\} \tag{6d}$$

All these expressions can be put in the form

$$\sum_\mu g_\mu(\mathbf{r})\cdot f_\mu(\mathbf{s}_1,\ \mathbf{s}_2,\ \mathbf{p})^* \tag{7}$$

where f_μ and g_μ are components of an "irreducible tensor." This "irreducible tensor" is a scalar in the expression (6*a*), a vector in (6*b*), a symmetric tensor of the second rank with trace zero in the second expression of (6*c*) and a tensor of the third rank in (6*d*). Thus, e.g.,

$$(\mathbf{r}\cdot\mathbf{s}_1)(\mathbf{r}\cdot\mathbf{s}_2) - {}^1/_3 r^2(\mathbf{s}_1\cdot\mathbf{s}_2) = xy(s_{1x}s_{2y} + s_{1y}s_{2x}) +$$
$$xz(s_{1x}s_{2z} + s_{1z}s_{2x}) + yz(s_{1y}s_{2z} + s_{1z}s_{2y}) + {}^1/_2(x^2 - y^2)(s_{1x}s_{2x} - s_{1y}s_{2y}) +$$
$${}^2/_3(z^2 - {}^1/_2x^2 - {}^1/_2y^2)(s_{1z}s_{2z} - {}^1/_2s_{1x}s_{2x} - {}^1/_2s_{1y}s_{2y})$$

On "time reversal" (condition 6) $\mathbf{r}$ remains unchanged while $\mathbf{s}_1$, $\mathbf{s}_2$, $\mathbf{p}$ go over into $-\mathbf{s}_1$, $-\mathbf{s}_2$, $-\mathbf{p}$ because the directions of all velocities are changed. This excludes, for the present, all types which are of odd order in $\mathbf{s}_1$, $\mathbf{s}_2$, $\mathbf{p}$, so that only the following types remain

$$\left.\begin{aligned} &1; \quad Q_{12}; \quad V_1 = (r\cdot[(\mathbf{s}_1 + \mathbf{s}_2) \times \mathbf{p}]) = ((\mathbf{s}_1 + \mathbf{s}_2)\cdot[\mathbf{p} \times \mathbf{r}]) \\ &t = (\mathbf{r}\cdot\mathbf{s}_1)(\mathbf{r}\cdot\mathbf{s}_2) - {}^1/_3 r^2(\mathbf{s}_1\cdot\mathbf{s}_2) \end{aligned}\right\} \quad (8a)$$

$$V_2 = ((\mathbf{s}_1 - \mathbf{s}_2)\cdot[\mathbf{p} \times \mathbf{r}]) \quad (8b)$$

All five expressions (8) are hermitean as they stand, and condition 2 will be satisfied if they are multiplied by a *real* function of r. (They all commute with any function of r.)

The enumeration of this section immediately gives the interactions between two particles of the same character (e.g., two protons). In this case there is, of course, no reason to introduce an isotopic spin. However, because of condition 7, the interaction V_2 must be eliminated. One sees, therefore, that in this case there are only the well-known ordinary (1), exchange (Q_{12}), vector (V_1), and tensor (t) interactions.

4. A formalism which does not use the isotopic spin would give the above four types of interactions between a pair of protons, the same types between a pair of neutrons and all five types (8) between a proton and a neutron—altogether 13 types. However, as Cassen and Condon[4] have noted, the use of the isotopic spin formalism permits a greater variety of interactions satisfying our conditions.

Because of condition 8, the interaction operator must commute with the operator of the total charge, $-{}^1/_2(\tau_{\xi 1} + \tau_{\xi 2} + \ldots + \tau_{\xi n}) + {}^1/_2 n$. Among the 16 matrices involving τ_1 and τ_2, the following have this property:

$$1; \quad {}^1/_2(\tau_{\xi 1} + \tau_{\xi 2}); \quad \tau_{\xi 1}\tau_{\xi 2}; \quad T_{12} = {}^1/_2 + {}^1/_2(\tau_1\cdot\tau_2), \quad (9a)$$

$$\tau_{\xi 1} - \tau_{\xi 2}, \quad (9b)$$

$$\tau_{\xi 1}\tau_{\eta 2} - \tau_{\eta 1}\tau_{\xi 2}. \quad (9c)$$

All the τ operators are, of course, invariant with respect to displacements, Galilei transformations, rotations and reflections so that the limitation of the interactions to the ten types (6) remains valid even if one multiplies these by any of the operators of (9). However, the time reversal involves a transition to the conjugate imaginary and only the interaction (9a) and (9b) remain invariant under this operation. Those of (9a) are, further-

more, symmetric in the particles 1 and 2 so that they can be combined immediately with the operators (8*a*), while $\tau_{\xi 1} - \tau_{\xi 2}$ can be combined only with (8*b*). On the other hand, $\tau_{\xi 1}\tau_{\eta 2} - \tau_{\eta 1}\tau_{\xi 2}$ changes sign upon time reversal and can be combined, therefore, with those operators of (6) which have the same property and which were eliminated for this reason from (8*a*) and (8*b*). Since, furthermore, $\tau_{\xi 1}\tau_{\eta 2} - \tau_{\eta 1}\tau_{\xi 2}$ is antisymmetric in the particles, condition 7 permits it to be combined only with those expressions in (6) which are also antisymmetric. Only

$$V_3 = (\mathbf{r}\cdot[\mathbf{w}^{(3)} \times \mathbf{p}]) = (\mathbf{w}^{(3)}\cdot[\mathbf{p} \times \mathbf{r}]) = ([\mathbf{s}_1 \times \mathbf{s}_2]\cdot[\mathbf{p} \times \mathbf{r}]) \quad (8c)$$

satisfies both conditions and this is therefore the only interaction that can be combined with $\tau_{\xi 1}\tau_{\eta 2} - \tau_{\eta 1}\tau_{\xi 2}$.

Our final result is therefore: There are 18 invariant types of interaction between pairs of particles which are linear (or of the zero'th order) in the momenta. Of these, 16 are obtained by multiplying any of the expressions (8*a*) with any of (9*a*), the last two by multiplying (8*b*) with (9*b*) and (8*c*) with (9*c*). All the expressions obtained in this way can be multiplied with an arbitrary real function of r. Combination of 1, Q_{12}, V_1, and t with the operators 1; $^1/_2(\tau_{\xi 1} + \tau_{\xi 2})$, $\tau_{\xi 1}\tau_{\xi 2}$, makes it possible to give the former operators different magnitudes and space dependences for proton-proton, proton-neutron and neutron-neutron pairs. The interaction $(\tau_{\xi 1} - \tau_{\xi 2})V_2$ corresponds to the operator V_2; it can act only between proton-neutron pairs. The operator T_{12} gives, with 1, Q_{12}, V_1, t, four interactions involving charge exchange which appear only in the formalism using isotopic spin. Similarly $(\tau_{\xi 1}\tau_{\eta 2} - \tau_{\eta 1}\tau_{\xi 2})V_3$ involves, because of

$$i(\tau_{\xi 1}\dot{\tau}_{\eta 2} - \tau_{\eta 1}\tau_{\xi 2}) = T_{12}(\tau_{\xi 1} - \tau_{\xi 2}) \quad (10)$$

a charge exchange.

5. We shall now briefly describe the physical properties of these 18 interactions. We shall first pick out those operators which can be given forms which (1) involve neither **s** nor τ, (2) may involve **s** but do not invole τ, (3) may involve τ but do not involve **s**. The corresponding interactions do not invalidate the relations derivable on the basis of approximations 1, 2 and 3, respectively, of reference 6. Second we shall inquire into the conditions under which (1) both spin and orbital angular momentum, (2) at least the orbital angular momentum, (3) at least the spin angular momentum, have definite values in the stationary states of a two particle system. It is customary to describe these situations by saying that the corresponding angular momentum quantum numbers are "good" quantum numbers.

It is relation (3) and the use of P_{12} for the exchange of space coördinates which makes it possible to write some of the operators in different ways and thus eliminate **s** or τ from some of the above types of interaction.

Approximation 1 will be valid if we have only ordinary and Majorana exchange forces

$$1 \text{ and } T_{12}Q_{12} = -P_{12} \tag{11a}$$

Approximation 2 permits ordinary, Majorana exchange, the so-called Heisenberg and Bartlett forces, as well as ordinary and exchange tensor and vector interactions: 1, $T_{12}Q_{12} = -P_{12}$, Q_{12}, t, V_1, $T_{12} = -Q_{12}P_{12}$, $T_{12}t$, $T_{12}V_1$. Approximation 3 will be valid if all the forces present are of the type 1, $T_{12}Q_{12} = -P_{12}$, $Q_{12} = -T_{12}P_{12}$, $^1/_2(\tau_{\xi 1} + \tau_{\xi 2})$, $^1/_2(\tau_{\xi 1} + \tau_{\xi 2})Q_{12} = -^1/_2(\tau_{\xi 1} + \tau_{\xi 2})P_{12}$, $\tau_{\xi 1}\tau_{\xi 2}$, $\tau_{\xi 1}\tau_{\xi 2}Q_{12} = (T_{12} - 1 - \tau_{\xi 1}\tau_{\xi 2})P_{12}$, T_{12}.

If one multiplies a permissible interaction operator with one of the twelve operators which can be derived from 1, Q_{12} or t of (8a), the resulting expression necessarily satisfies all conditions for permissible operators, except condition 2 of section 2. Even this condition will be satisfied if one uses the symmetrized product instead of the simple product. In fact one can verify that all the 150 products obtained in this way are linear combinations of expressions of the 18 types given above. In particular, it is interesting to remark that the symmetrized products of the six expressions (8) with Q_{12} are $Q_{12}1 = Q_{12}$, $Q_{12}Q_{12} = 1$, $Q_{12}V_1 = V_1$, $Q_{12}t = t$, and $^1/_2(Q_{12}V_2 + V_2Q_{12}) = ^1/_2(Q_{12}V_3 + V_3Q_{12}) = 0$. These last equations follow from

$$Q_{12}V_2 = -V_2Q_{12} = iV_3; \quad Q_{12}V_3 = -V_3Q_{12} = -iV_2 \tag{10a}$$

On the other hand, $Q_{12}T_{12} = -P_{12}$ commutes with all permissible interactions. One can obtain the product of P_{12} and any permissible interaction by means of (10a) and the further relations

$$\left.\begin{aligned} &T_{12}{}^1/_2(\tau_{\xi 1} + \tau_{\xi 2}) = {}^1/_2(\tau_{\xi 1} + \tau_{\xi 2}); \; T_{12}\tau_{\xi 1}\tau_{\xi 2} = 1 + \tau_{\xi 1}\tau_{\xi 2} - T_{12}; \; T_{12}{}^2 = 1 \\ &T_{12}(\tau_{\xi 1} - \tau_{\xi 2}) = i(\tau_{\xi 1}\tau_{\eta 2} - \tau_{\eta 1}\tau_{\xi 2}); \; T_{12}(\tau_{\xi 1}\tau_{\eta 2} - \tau_{\eta 2}\tau_{\xi 1}) = -i(\tau_{\xi 1} - \tau_{\xi 2}) \end{aligned}\right\} \tag{10b}$$

Whether or not the orbital and spin angular momentum quantum numbers are good quantum numbers for the description of states of two particle systems is determined by the commutation properties of the interaction between the two particles with the operators $[\mathbf{p} \times \mathbf{r}]^2 = \mathbf{L}^2$ and $(\mathbf{s}_1 + \mathbf{s}_2)^2 = \mathbf{S}^2$ for the relative orbital and spin angular momentum, respectively. Since neither of these operators involves τ, the commutation properties of all 18 types of interaction are determined by the properties of the six interactions (8a, b, c).

That $\mathbf{L}^2$ commutes with 1 and Q_{12} is obvious. V_1, V_2 and V_3 can all be written as the dot product of a spin operator with $\mathbf{L}$; since $\mathbf{L}^2$ commutes with all components of $\mathbf{L}$ it commutes with V_1, V_2, V_3.[†] The only remaining operator, t, does not commute with $\mathbf{L}^2$.

The discussion of the commutation with $\mathbf{S}^2$ reduces to that of the commutation with Q_{12} for $\mathbf{S}^2 = 4(1 + Q_{12})$. Since Q_{12} is the operator which

interchanges $\mathbf{s}_1$ with $\mathbf{s}_2$ it clearly commutes with 1, Q_{12}, V_1, t, since these are symmetric in the spins, and anticommutes with the V_2 and V_3 which are antisymmetric in the spins.

The eigenvalues of the orbital and spin operators are both good quantum numbers for the two particle system if the interaction between the particles consists of a combination of types involving 1, Q_{12} and V_1, since these interactions commute with $\mathbf{L}^2$ and $\mathbf{S}^2$. If a term of type t is included in the interaction the orbital angular momentum no longer gives a good quantum number; likewise, if in addition to 1, Q_{12}, V_1, the interaction includes V_2 or V_3 the spin quantum number is bad. Finally if the interaction includes both t and V_1 or V_2 the only good quantum numbers are those demanded by the symmetry properties of space-time. It is remarkable that only the two relatively complicated interactions $V_2(\tau_{\xi 1} - \tau_{\xi 2})$ and $V_3(\tau_{\xi 1}\tau_{\eta 2} - \tau_{\eta 1}\tau_{\xi 2})$ (which do not contribute to the interaction between two protons or two neutrons) spoil the spin eigenvalues as quantum numbers and thus lead to a mixing of $^3P_1$ and $^1P_1$, and of similar pairs of states.

* Throughout this paper h stands for (Planck's constant)/2π, i.e., Dirac's h.

† It should be noted that $\mathbf{L} = [\mathbf{p} \times \mathbf{r}]$ is the orbital momentum only for a two particle system. The total orbital momentum operator for a system with more than two particles does not commute with V_1, V_2, V_3.

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RIBOFLAVIN CONTENTS OF TISSUES AS STABILIZED IN THE ADULT AT LIBERAL LEVELS OF INTAKE

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Earlier experiments in our own and other laboratories[1, 2, 3] showed that the tissues of animals normally nourished contain more riboflavin than do corresponding tissues of animals which have for some time been fed diets practically devoid of riboflavin. In more recent experiments of Ellis and Zmachinsky in this laboratory (carried out with the aid of a grant from the Carnegie Corporation of New York through the Carnegie Institution of Washington), it has been found that this difference in tissue content of riboflavin dependent upon the level of intake is clearly demonstrable as be-

tween animals reared on a minimal-adequate allowance of riboflavin and those fed half as much.

Meanwhile it had also been found[4, 5] that nutritional well-being continues to be enhanced by successive increases of riboflavin intake up to levels more than twice that of minimal adequacy.

On submitting to bioassay[6] the tissues of adult rats which had received and profited by these more-than-merely-adequate intakes of riboflavin, we did not obtain conclusive evidence that these further increases of intake had further raised the concentration of riboflavin in the tissues.

One of us (F. V. D.) therefore undertook a further investigation of the quantitative determination of the riboflavin of tissues, and has developed a new method for such determination *in vitro* which is described and discussed in another paper.[6] Examined by this new *in vitro* method, tissues of rats reared on food containing approximately 5 and 20 micrograms of riboflavin per gram of food showed, respectively: Liver, 39.6 ± 0.35 and 40.8 ± 0.60; kidney, 34.8 ± 0.76 and 33.6 ± 0.79; heart, 27.2 ± 0.47 and 26.6 ± 0.64; skeletal muscle (by bioassay) 3.9 ± 0.29 and 3.4 ± 0.30 micrograms of riboflavin per gram of fresh tissue. These values are higher than those recorded by previous investigators. The extent to which this may be due to differences in method is discussed elsewhere.[6] *In vitro* measurements for skeletal muscle were less satisfactory, because the extracts here obtained had a slightly different color fluorescence. Bioassays showed no significant difference in the riboflavin contents of the skeletal muscles, and only a doubtful difference, if any, in the other tissues of the animals fed at these different levels. From the evidence as a whole it seems safe to conclude that the tissues of the animals on both these liberal levels of intake were of stabilized maximal riboflavin content.

As the rats on the higher of these two liberal levels seem to produce young of greater vitality,[5, 7] it would appear that when tissue content of riboflavin has reached its "plateau" and does not rise appreciably further in response to still higher levels of intake, such higher intakes may nevertheless improve the body's internal environment in some other way, thus giving rise to more vigorous offspring.

The further question now arises whether these increments of intake above the level at which the concentration in adult tissue is stabilized may still result in a higher riboflavin content of the offspring at birth or during infancy, or both. It is hoped that this new question may be studied in later experiments.

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THE PHYSICO-CHEMICAL STRUCTURE OF THE SALIVARY GLAND CHROMOSOMES. II

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It was evident from the experiments previously reported[1] that upon treating the salivary gland chromosome with aqueous alkali a sequence of changes was instituted which involved more than one chemical reaction. Some of these reactions might be parallel (alternative) and others might be consecutive. One method by which these several reactions might be controlled and their effects separated one from another so as to be recognizable as products of simpler reactions is to perform the experiments at different temperatures. Thus, since the several reactions almost certainly have different temperature coefficients, their relative rates will be changed and the resulting product may be quite different, or one step may appear, more clearly separated from the others. This is indeed the case as the following experiments show.

Temperature Effect on the Chromosome Changes Produced by Alkali. Experimental.—The technique of the treatment and staining is the same as that already described. Experiments were carried out at two temperatures, 25°C. and 10°C. In the former set of experiments (25°C.), glands were treated with standardized 1 *N* NaOH for varying lengths of time from 1 min. to 11 min. in a chamber kept constantly at 25°C. For the experiments at 10°C., a chamber constantly maintained at this temperature was employed. Glands, glassware and reagents were cooled in the chamber several minutes and then 1 *N* NaOH was applied to the glands for the desired length of time. In both sets of experiments, the number of nuclei was recorded according to their structure, either the normal, the granule or the chromatid stage. These stages are shown in figure 1. Figure 1 (*a*) represents the normal chromosome, figure 1 (*b*) represents the form called the granule stage and figures 1 (*c*) to 1 (*h*), inclusive, represent all of the forms which have been counted as the chromatid stage. This grouping together of all structures in which some form of longitudinal chromatic string

is visible was done because in these experiments no sharp line could be drawn between them. They represent a more or less continuous transition

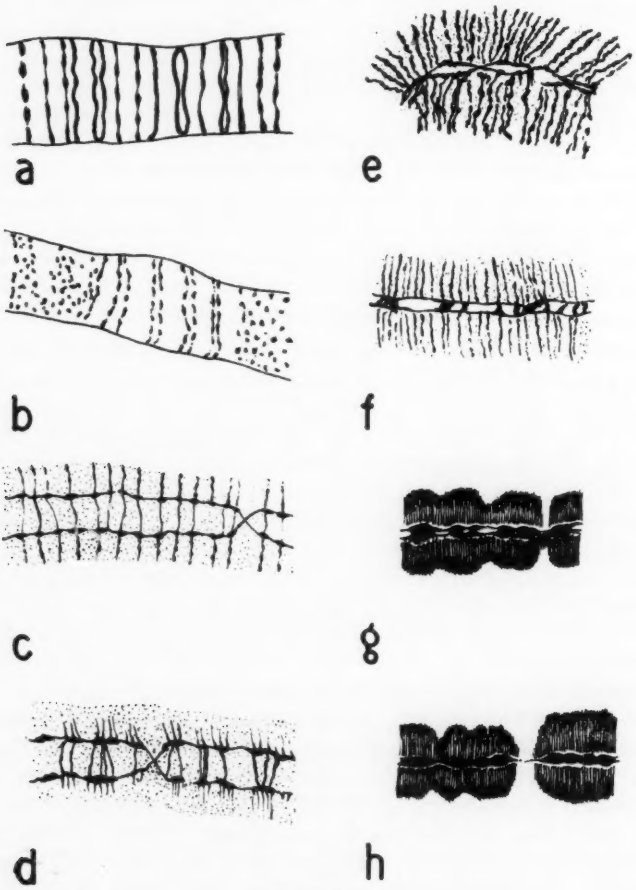

FIGURE 1

The semidiagrammatic drawings of the structures of salivary chromosome after treatment with various reagents as described in the text. *a*, Normal structure; *b*, granule stage; *c*, *d*, *e*, *f*, *g* and *h* are the various structures of the chromatid stage.

in which the central string becomes more clearly defined with a concomitant definition of the hairs. The results of these experiments are summarized in table 1.

TABLE 1

THE EFFECT OF 1 *N* NaOH ON THE SALIVARY GLAND CHROMOSOME AT TWO TEMPERATURES (10°C. AND 25°C.)

TIME IN MINUTES	NORMAL STAGE NO. OF NUCLEI	%	GRANULE STAGE NO. OF NUCLEI	%	CHROMATID STAGE NO. OF NUCLEI	%	TOTAL NO. OF NUCLEI
			25°C.				
1	7	0.9	654	81.1	145	18.0	806
2	5	0.5	517	55.2	414	44.2	936
3	13	1.3	295	30.1	673	68.6	981
4	4	0.8	138	26.4	381	72.8	523
5	3	0.4	141	20.2	554	79.4	698
6	1	0.1	124	12.1	902	87.8	1027
7	1	0.2	49	9.4	468	90.3	518
8	0	0	24	4.2	551	95.8	575
9	0	0	29	3.3	850	96.7	879
10	0	0	23	2.7	1034	97.3	1063
11	0	0	4	0.3	1182	99.7	1186
			10°C.				
1	1	0.2	599	94.5	34	5.4	634
2	15	1.7	629	69.4	262	28.9	906
3	10	1.7	372	63.4	205	34.9	587
4	6	0.9	287	44.3	355	54.8	648
5	1	0.2	212	38.5	338	61.3	551
6	0	0	161	34.5	306	65.5	467
7	0	0	77	25.0	231	75.0	308
8	0	0	168	24.6	516	75.4	684
9	0	0	82	19.7	335	80.3	417
10	0	0	71	17.8	327	82.2	398
11	0	0	46	13.5	294	86.5	340

Results.—In order to interpret the results presented in table 1 more quantitatively let us first consider the general case of a pair of consecutive irreversible reactions (unimolecular or pseudounimolecular) represented by

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C \qquad (1)$$

where k_1 and k_2 are the unimolecular velocity constants for the reactions $A \longrightarrow B$ and $B \longrightarrow C$, respectively. The differential rate laws for this system are

$$\frac{d[A]}{dt} = -k_1[A] \qquad (2)$$

$$\frac{d[B]}{dt} = k_1[A] - k_2[B] \qquad (3)$$

$$\frac{d[C]}{dt} = k_2[B] \qquad (4)$$

which give upon integration the following relationships.

$$\frac{[A]}{A_0} = e^{-k_1 t} \tag{5}$$

$$\frac{[B]}{A_0} = \frac{k_1}{k_2 - k_1}\left(e^{-k_1 t} - e^{-k_2 t}\right) \tag{6}$$

$$\frac{[C]}{A_0} = 1 - \frac{k_2}{k_2 - k_1} e^{-k_1 t} + \frac{k_1}{k_2 - k_1} e^{-k_2 t} \tag{7}$$

Since A_0 is the total number of units in the original unchanged form A at the start of the reaction (time, t, is zero), $[A]/A_0$ is the fraction (or percentage) of the total number of units remaining in the form A at the time t, $[B]/A_0$ is the fraction (or percentage) of the total number of units in the form B at the time t and $[C]/A_0$ is the fraction (or percentage) of the total number of units in the form C at the time t.

The application of the above relationships to the chromosomes follows quite readily if we let A represent the initial unchanged chromosomes, B the chromosomes in the granule stage and C the chromosomes in the aggregate of stages called the chromatid stage in the table. It is evident that the transition from the normal stage to the granule stage proceeds considerably more rapidly than the transition from the granule stage to the chromatid stage, i.e., $k_1 > k_2$. Allowing this approximation, equations (6) and (7) become

$$\frac{[B]}{A_0} = e^{-k_2 t} \tag{6a}$$

$$\frac{[C]}{A_0} = 1 - e^{-k_2 t} \tag{7a}$$

Now $[A]/A_0$, $[B]/A_0$ and $[C]/A_0$ are just the percentages given in the columns of table 1 under "normal stage," "granule stage" and "chromatid stage," respectively. The values given for $[A]/A_0$ are in far too small a range to be tested by equation (5), the individual experimental errors in each point being of the same order of magnitude as the entire range. The values for $[B]/A_0$ or $[C]/A_0$, however, extend over a wide range and can be tested by equation (6a) or (7a), respectively. This we have done in figure 2 in which the logarithm of the % in the granule stage (log $[B]/A_0$) is plotted as ordinate and the time is plotted as abscissa, for the two temperatures, 10°C. and 25°C. It is evident that the approach of the points to a straight line is satisfactory for this type of statistical experiment.

The slopes of the lines in figure 2 are a measure of k_2 at each of the two temperatures. The temperature coefficient of the velocity constant may be

expressed in terms of the activation energy of the reaction, which is given by

$$E = R \frac{TT'}{T - T'} \ln \frac{k}{k'} \tag{8}$$

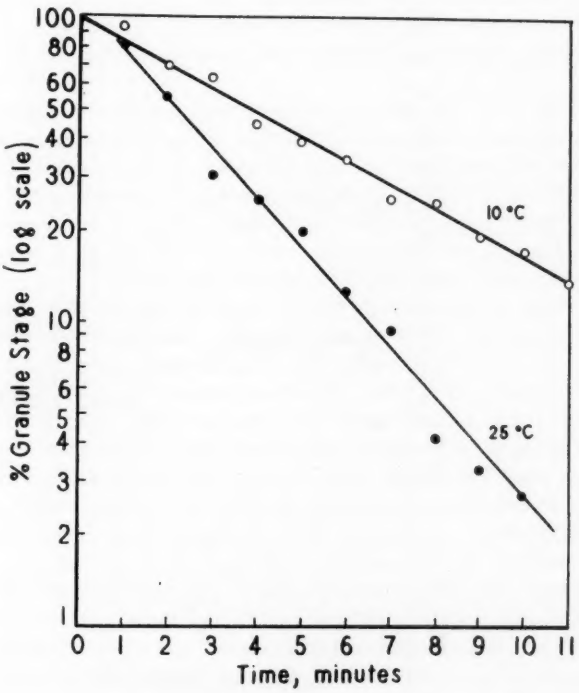

FIGURE 2

Semi-logarithmic plot of the data of table 1.

For k_2, from the lines of figure 1, the activation energy is

$$E_2 = 4.59 \frac{298 \times 283}{15} \log 2.07$$

$$\cong 8 \text{ Kcals.}$$

From the fact that $[A]/A_0$ is practically the same at both temperatures we can quite safely conclude that the activation energy for k_1 must be less than 4 Kcals. and probably very close to zero. The 25° line apparently

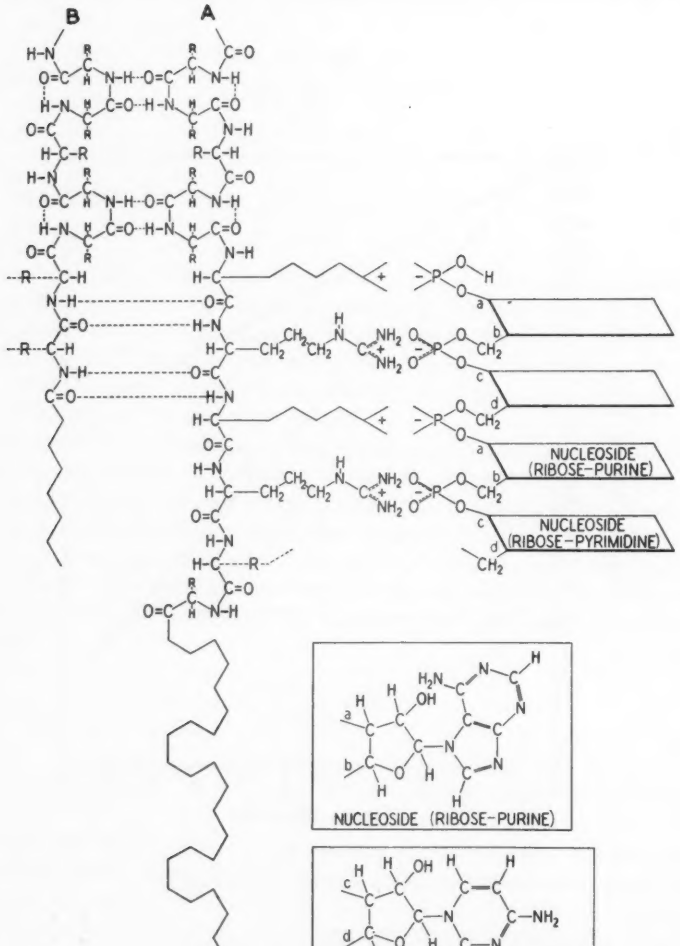
B
A
NUCLEOSIDE (RIBOSE-PURINE)
NUCLEOSIDE (RIBOSE-PYRIMIDINE)
NUCLEOSIDE (RIBOSE-PURINE)
NUCLEOSIDE (RIBOSE-PYRIMIDINE)

FIGURE 3

does not go through the 100% value at zero time. This must be attributed to the neglected term in k_1 of equation (6), for at the higher temperature the value of k_2 is not as much smaller than k_1 as it is at the lower temperature.

Discussion.—At this point it does not seem too presumptuous to us to record in somewhat more detail the conception of chromosome structure to which we have been led by the experiments so far described in this and the previous publica[illegible] together with results and suggestions of other workers referred to therein, and which has led us in the design of further experiments.

In figure 3 is given a diagram of what is probably the essential unit in the construction of a chromosome of the type we are discussing. Its most important elements are the partially extended and partially folded polypeptide chain, together with the fitting of the nucleic acid onto the extended portion of the chain. This fitting together of the nucleic acid and the extended polypeptide chain was suggested by Astbury and Bell[2] on the basis of some x-ray measurements which gave very nearly the identical periodicity of 3.4 A along the fibre axis, for clupein, sodium thymonucleate and clupein thymonucleate. The flat parts of the nucleic acid molecules, pyrimidine and purine nuclei, have their planes parallel to each other (at a distance of 3.4 A) and normal to the direction of the main polypeptide chain to which they are attached. The chain marked *A* in the figure is only one of a bundle of such chains tied together by ordinary covalent bonds through the side chains *R*, into a three-dimensional pattern. Chain *B* is one chain of another such bundle of primarily connected chains. The forces which hold chains *A* and chains *B* together are, however, of a much weaker type and probably primarily hydrogen-bonds between the NH groups and the C=O groups. These hydrogen-bonds are represented by the dotted lines.[3] A number of such parallel bundles held in place by these comparatively weak bonds are thus supposed to constitute the chromosome, the transverse chromatic bands being the coalescence of the nucleic acid rich regions along the extended portion of the polypeptide chains and the interchromatic regions being the highly folded portions of

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EXPLANATION OF FIGURE 3

The chain *B* represents a typical polypeptide chain partially folded and partially extended. In the upper portion the atoms of the chain are given in detail, the lower part of the figure being simply an outline of the form. The letter *R* represents the particular residue associated with the several different amino acids which constitute the chain. In the case of nucleo-protein these residues are mostly arginine of which a few are drawn out in detail on the middle stretched part of the chain. Attached to these arginine residues by means of a salt link (represented by + and − signs) between the basic guanidine group and the phosphoric acid of the nucleotide (nucleic acid) is a tetranucleotide molecule. There are only four nucleoside residues shown in the figure corresponding to the suggestion of the structure of thymonucleic acid, but actually there is undoubtedly a large variety of groupings. For a further description of this figure see the text.

the polypeptide chain comparatively poor in nucleic acid. It should be borne in mind that the simple folding shown in the figure can hardly be the entire picture. There almost certainly exist secondary and more complex folding of entire bundles of chains as well.

On the basis of this model, the initial step, i.e., the formation of the granule stage, with a very small temperature coefficient, is to be interpreted as a neutralization reaction followed by some diffusion and condensation of the nucleic acid residues. The neutralization involves the breaking of the hydrogen-bonds to the C=O by the attachment of OH^- to the

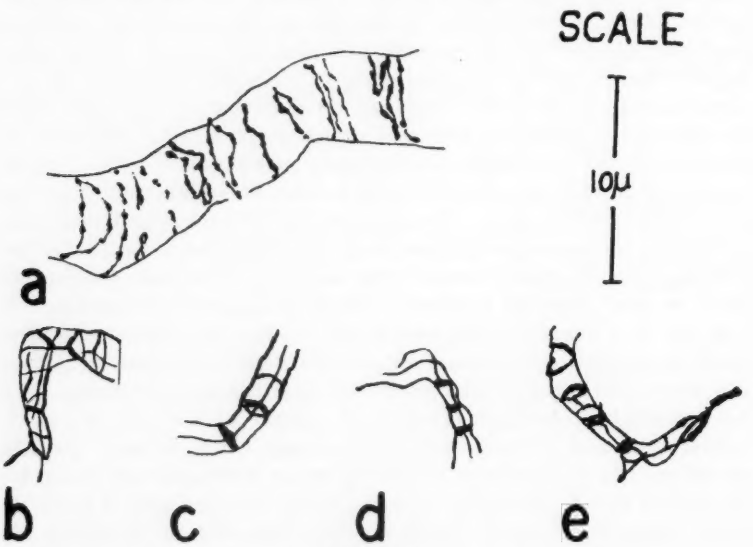

FIGURE 4

The camera lucida drawings of the granule and chromatid stages produced by treatment with urea and urea-NaCl combination. *a*, Granule stage produced with urea; *b*, *c*, *d* and *e*, the chromatid stage produced with urea-NaCl mixture or with urea followed by NaCl.

acidic proton of the amide group. Thus with these restraining forces released (not all types of restraining forces) the flat nucleic acid residues acquire a certain amount of freedom and they condense in groups in so far as they are able. This process of condensation has been observed with sodium thymonucleate[4] as well as with other types of flat molecules. The next step, having an apparent activation energy of about 8 Kcals., must involve the breaking of stronger bonds, with the result of a more complete freedom for the nucleic acid residues allowing them to rearrange and condense into the fairly long strings of the chromatid stage. We do not

yet know what these other bonds are. They may be sulfide links, esters (of both carboxylic and phosphoric acids), phosphoric acid amides or peptide (carboxylic acid amides) links. In this connection it is of interest to note that among alkaline hydrolyses the activation energy for that of diketopiperazine, 6.5 Kcals.,[5] best approaches the value, 8 Kcals., we have here reported. This correspondence is not to be taken too seriously since the value of 8 Kcals. is certainly not that of a simple reaction. It may include not only a number of reaction rates but also preliminary ionization equilibria.

There is another recent observation which may have some bearing on the present problem and that is the calorimetric observation that the alkaline denaturation (pH 10–12) of methemoglobin involves at least two reactions, the first of which absorbs heat and the second evolves heat.[6]

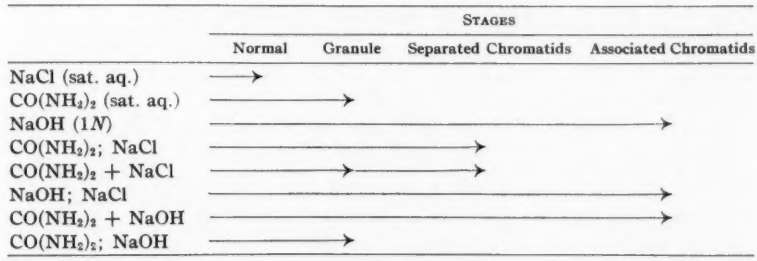

FIGURE 5

Effects produced by various reagents on the salivary gland chromosome. The stages occur in the sequence given from left to right and arrows indicate the final structure attainable with each reagent previous to total solution. When more than one reagent is used, the semicolon indicates consecutive application of the reagents while the plus sign indicates simultaneous application.

This implies that the entropy change for the initial reaction is positive, i.e., involves an increase in probability, such as would actually be the case in a disorganization reaction which is here suggested.

Chromosome Changes Produced by Urea.—If the suggestions we have just outlined are correct, it should be possible to find reagents which would produce the first step without inducing the second. One such reagent is urea which has been shown to be capable of breaking hydrogen-bonds.[7] It should not, however, be able to produce the effects of the second alkali reaction, i.e., the one having the apparent activation energy of 8 Kcals. This was indeed found to be so. Glands were treated with a saturated aqueous solution of urea for varying lengths of time at room temperature. Within a few minutes after the application of the urea solution, the chromosomes began to swell with simultaneous granulation of the chromatic

bands. As the time of treatment increased the chromatic granules moved laterally and separated further and further apart from each other. No other apparent changes were observed. (See Fig. 4 (*a*).)

Since salt is an effective condensing agent for the flat molecules of which we have spoken[1] the effect of NaCl solutions on the urea-treated chromosomes as well as the alkali-treated chromosomes was observed. Glands were first immersed in a saturated aqueous urea solution for thirty minutes and treated with saturated aqueous NaCl for five minutes. The resulting structures are shown in figure 4 (*b*, *c*, *d* and *e*). The same results are noted when the urea and NaCl solutions are applied simultaneously. It should be noted that no matter how long this treatment is applied (30 minutes) the chromatic strings do not associate to give a uniform structure such as figure 1 (*h*). The structure of figure 4 (*b*, *c*, *d* and *e*) we called the separated chromatid structure in contrast with the associated chromatid structure (Fig. 1 (*c*, *d*, *e*, *f*, *g*, *h*)). The separated chromatid stage is the one in which the stronger, alkali susceptible bonds have not been broken. If, however, the glands are treated with solutions containing both urea and NaOH the associated chromatid stages always appear.

The effect of NaCl on the alkali-treated chromosomes is to accelerate the condensation process. Glands were first immersed in 1 *N* NaOH for 5 minutes and then treated with a saturated aqueous solution of NaCl for 5 minutes. The number of nuclei in the chromatid stage was counted. Of the 503 nuclei which were treated all of them appeared in the chromatid stage. However, in table 1 the percentage of the chromatid stage produced by treatment with 1 *N* NaOH only for 5 minutes is 79.4%. Hence it is evident that the condensation is increased by NaCl.

The effects of the various reagents used are summarized in figure 5.

* Services rendered by the personnel of The Work Projects Administration Official Project No. 165-1-08-73 are hereby acknowledged.

[1] Calvin, M., Kodani, M., and Goldschmidt, R., *Proc. Nat. Acad. Sci.*, **26**, 340 (1940).

[2] Astbury, W. T., and Bell, F. O., *Cold Spr. Har. Sym. on Quant. Biol.*, **6**, 109–121 (1938). See reference 1 for a more complete discussion of how this type of model can be used to interpret a number of other physical and chemical phenomena that have been described for these chromosomes.

[3] For a discussion of hydrogen-bonds in proteins see (*a*) Astbury, W. T., *Trans. Faraday Soc.*, **36**, 871 (1940), (*b*) Buswell, A. M., Krebs, K. F., and Rodebush, W. H., *Jour. Phys. Chem.*, **44**, 1126 (1940), (*c*) Pauling, L., and Niemann, C., *Jour. Am. Chem. Soc.*, **61**, 1860 (1939).

[4] Signer, R., Caspersson, T., and Hammarsten, E., *Nature*, **141**, 122 (1938). For similar observations on other substances see reference 1.

[5] Kuhn, W., Molster, C. C., and Freudenberg, K., *Ber.*, **65**, 1179 (1932).

[6] Conn, J. B., Kistiakowsky, G. B., and Roberts, R. M., *Jour. Am. Chem. Soc.*, **62**, 1895 (1940).

[7] Buswell, A. M., Krebs, K. F., and Rodebush, W. H., *Jour. Phys. Chem.*, **44**, 1126 (1940).

CERTAIN SINGULAR POINTS ON CRYSTALLIZATION CURVES OF SOLID SOLUTIONS

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Many ternary systems have been investigated in which two of the components form a complete series of solid solutions of the type with a minimum melting temperature, while each of them forms a eutectic with the third component as in figure 1. In spite of this fact there is great diversity of opinion as to the theoretical courses of curves of crystallization in such a system, and the experimental work has in no instance been sufficiently accurate to establish a basis for acceptance or rejection of any of the views.

Schreinemakers laid the foundation for the theoretical treatment by considering the relations at various isothermals with the aid of Gibbs Zeta function.[1] In figure 2 is given an isothermal diagram for a temperature higher than the minimum temperature in the solid solution series *A–B*. There are here three series of saturated liquids, the first series in equilibrium with solid solutions rich in *A*, the second with solid solutions rich in *B* and the third with the pure substance *C*. A number of tie lines are shown on the figure which join the point representing the composition of a liquid with the point representing the composition of the crystals in equilibrium with it. In the one corner of the triangle these are simply lines radiating from *C* and have no properties requiring special notice here. In the other two corners it will be observed that each liquid is in equilibrium with a different solid solution.

Figure 3 shows equilibrium for a temperature lower than the minimum in the system *A–B*. Here there are but two series of saturated liquids. Of one series each member is in equilibrium with the pure substance *C*. Of the other series each is in equilibrium with a distinct solid solution. Now since a liquid from which any solid is crystallizing and in which no other influence is operative must change in composition directly away from the composition of that solid, the direction of instantaneous change of composition of a saturated liquid at any temperature is given by its appropriate tie line. But, as the diagrams show, the composition of the solid solution in equilibrium with any liquid changes as the liquid changes in composition with falling temperature. Therefore the lines representing the change of composition of the liquid with fractional crystallization will be curved lines which, for any liquid at any temperature, will be tangent to the tie line for that liquid at that temperature. To illustrate this tangent relation there are shown in figures 2 and 3 short segments of typical curves of frac-

tional crystallization with falling temperature, although all other features of the diagrams are isothermal.

It is plain that if we have a large number of isothermal diagrams with tie lines we can construct the curves of fractional crystallization, and in the solid solution field, for the type of system under consideration, they have the general character shown in figure 4. They thus consist of two bundles of curves, one emanating from A and the other from B, separated from each other by a curve mf_1 which comes from the binary minimum m and which is itself also a curve of fractional crystallization. Schreinemakers carried the consideration of a system of this type to the point of calling attention to this general disposition of fractionation curves but apparently did not pursue it further in any later publication.

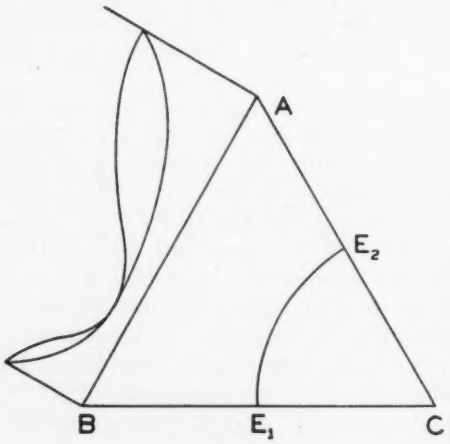

FIGURE 1

Equilibrium diagram of the system A–B–C and the system A–B.

Relation of Fractional Crystallization to Equilibrium Crystallization.—These curves represent the change of composition of a liquid when it is inert to crystals already separated, and a liquid which had experienced partial crystallization by this method would contain in it crystals of the solid solution phase (mix-crystals) of different compositions, the composition of the crystals forming at any instant (temperature) being given as we have seen by the tangent to the curve at that temperature. The presence of crystals of different composition represents failure of true equilibrium. For some purposes we are interested in the establishment of true equilibrium which is realized if the liquid, as it precipitates mix-crystals of the composition in equilibrium with it at any instant, at the same time changes the composition of all mix-crystals already present.

The course of a liquid during perfect equilibrium crystallization is therefore quite different from that during perfect fractional crystallization. If we have curves of fractional crystallization such as those shown in figure 4 we can derive from them the curves of equilibrium crystallization by a simple construction, the principle of which will appear if we return to a consideration of the isothermal figure 3. At the temperature of that iso-

therm any mixture of total composition lying on a tie line such as ls will consist of crystals of composition s and liquid of composition l. Therefore the point l will be a point on the curve of equilibrium crystallization of such mixtures as x and y. Remembering that the line ls is tangent at l to the curve of fractional crystallization (broken curve), and that the figure refers to any random temperature, we may readily conclude that points on the curve of equilibrium crystallization of any liquid x can be determined by dropping tangents from the point x to successive members of the bundle of curves of fractional crystallization. Thus in figure 5 the tangents xt_1 to Af_5, xt_2 to Af_6 and xf_7 to Af_7 determine points on the curve of equilibrium crystallization $xt_1t_2f_7$, which must end at the boundary curve E_1E_2, and s_7f_7 is therefore a three-phase boundary.[2]

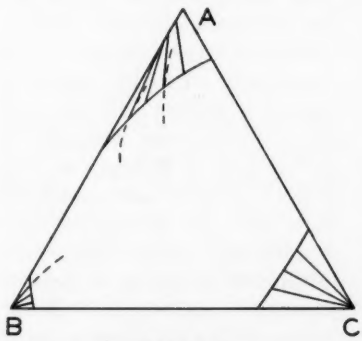

FIGURE 2

Isothermal diagram for a temperature above the binary minimum in the system A–B.

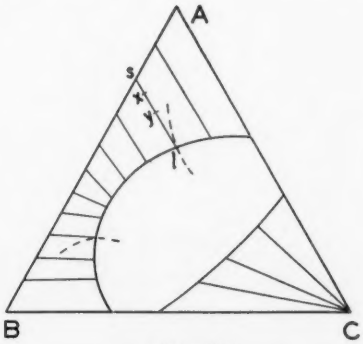

FIGURE 3

Isothermal diagram for a temperature below the minimum in the system A–B.

The importance of this simple construction whereby equilibrium curves can be derived from fractionation curves has not been fully appreciated, especially in its application to more complex cases. For example, in figure 4 most of the curves have the simple relation of the group that has been selected for constructing figure 5. But, since the fractionation curve coming from m will ordinarily not be a straight line, but will be at least somewhat curved, the adjacent curves of fractional crystallization on its convex side will necessarily be reversed curves. It is to the curves of equilibrium crystallization in this region that attention is here especially directed, and upon which there is so much difference of opinion. It is ordinarily assumed in papers on the subject that the curves of equilibrium crystallization for liquids close to the central fractionation curve on the A side would be reversed curves, with their points of inflexion on the central fractionation curve. No basis is offered for the assumption, indeed it has no basis.

We may develop curves of equilibrium crystallization from fractionation curves in any part of the diagram by applying the tangent relation already discussed. In figure 6 are shown three reversed curves of the bundle emanating from A, viz. Af_1, Af_2 and Af_3. If we drop tangents from the point x upon these curves we find that in general there are two points of tangency on each curve. Thus xt_1 and xt_5 are tangent to Af_1 at t_1 and t_5, xt_2 and xt_4 are tangent to Af_2 at t_2 and t_4. But, as a ray from x rotates anticlockwise and assumes the successive positions xt_1, xt_5, xt_2, xt_4, it finally comes to the position xt_3, where the two points of tangency coincide with each other and with the inflexion point t_3 of the curve Af_3. The rotating ray can have no point of tangency with a curve to the right of Af_3. It can only cut such curves. The curve $xt_1t_2t_3t_4t_5$ is therefore the curve of equilibrium crystallization of the mixture x. It experiences an abrupt change of direction and sign of curvature at t_3, which may be called a corner point. It might be supposed, without close consideration, that this derived curve of equilibrium crystallization would experience a change of sign of curvature without an abrupt change of direction, that is, would itself have a point of inflexion at t_3, but two such curves with a common point of inflexion must cross each other at that point. The supposed curve would therefore have to cross Af_3 at t_3, but we have already seen that there can be no points to the right of Af_3 having the same properties as t_1, t_2, etc., with respect to the initial point x (original liquid).

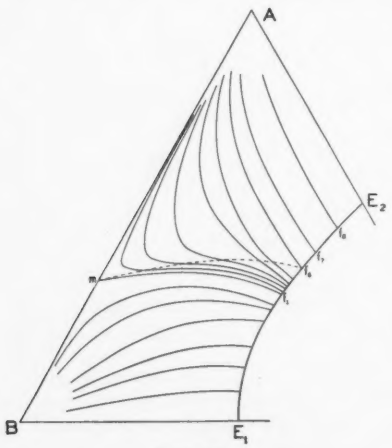

FIGURE 4

Curves of fractional crystallization in the field of solid solutions (full curves). Locus of points of inflexion (broken curve).

Through the points of inflexion of the fractionation curves of figure 4 the curve mf_6 has been drawn. This curve will also be the locus of corner points on curves of equilibrium crystallization. If points on this curve mf_6, representing as they do the composition of certain liquids, are joined, each with the solid solution in equilibrium with it (tie line), as has been done in figure 7, we obtain a diagram of service in considering the crystallization of any liquid which has a corner point on its curve of equilibrium crystallization. All liquids lying within the area ms_6f_6 will show this phenomenon and the tie lines enable us to state at what point on the

curve mf_6 the corner point will lie for any initial composition of the liquid. Thus any liquid on the join s_5i_5 will have a corner point on its curve of equilibrium crystallization at i_5, those on s_2i_2 at i_2 and so on for any tie line.

The Actual Process of Crystallization.—While the geometrical relations at an inflexion point on a fractionation curve are thus such as to require corner points on equilibrium curves, it is not in geometrical relations as such that primary interest lies. It is rather in the process of crystallization, and we therefore turn to the question of the actual physical significance of the corner points in this process. First it is desirable to lay a

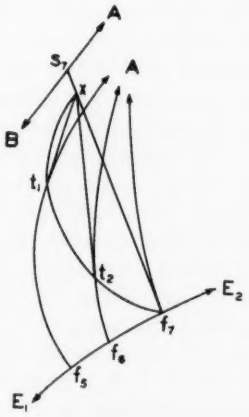

FIGURE 5

Derivation of curve of perfect equilibrium crystallization ($xt_1t_2f_7$) from curves of fractional crystallization Af_5, Af_6 and Af_7.

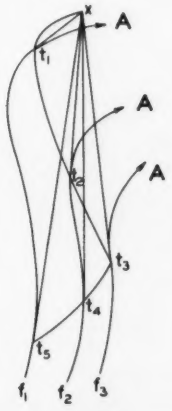

FIGURE 6

Derivation of curve of perfect equilibrium crystallization ($xt_1t_2t_3t_4t_5$) from curves of fractional crystallization Af_1, Af_2, Af_3.

foundation by considering the physical significance of simpler points. In figure 8 is shown the curve of fractional crystallization Ayf which passes through the point y in the field of the solid solutions of A and B. The tangent ys fixes the composition of the solid solution s in equilibrium with the liquid y, and yf is the course of *fractional crystallization* of the liquid y. At the temperature of the point y, and at equilibrium, all mixtures on the line sy will consist of crystals of composition s and liquid of composition y. Thus the liquid x will, with perfect *equilibrium crystallization*, follow the curve xny which cuts Ayf at y. Now it is, of necessity, true of any curve which represents the change of composition of a liquid during crystallization, that the tangent to the curve at any point must pass through

the composition of the crystalline material that is being substracted from the liquid at the point. The tangent ys_1 to the curve $xnyz$ at y thus fixes at s_1 the composition of the crystalline material being subtracted from the liquid y during the perfect equilibrium crystallization of the initial liquid x. Nevertheless the liquid y is in equilibrium with the crystals s. The reason for the change of composition of the liquid y in the direction s_1y, during perfect equilibrium crystallization, is that this type of crystallization is compounded of two effects. As a result of pure subtraction of new crystals the liquid y tends to move an infinitesimal distance along the curve yf for an infinitesimal drop of temperature, and at the same time the composition of the crystals separating moves from s to a point an infinitesimal distance towards B. This is the purely fractionation effect, but there is another effect. The liquid y, having come into being as the result of equilibrium crystallization of liquid x, contains a large quantity of crystals of s, indeed an amount about equal to the amount of liquid. During perfect equilibrium crystallization, occasioned by an infinitesimal fall of temperature at the temperature y, this finite amount of crystals requires to be modified in composition from s to a point an infinitesimal distance towards B from s, in short the crystals must be enriched in B. The only source of B is the liquid, therefore the crystals already present subtract B from the liquid, which effect, taken by itself, would cause the liquid to change directly away from B. The actual movement is compounded of these two effects. The liquid thus moves in a direction away from a point between s and B, namely, s_1.

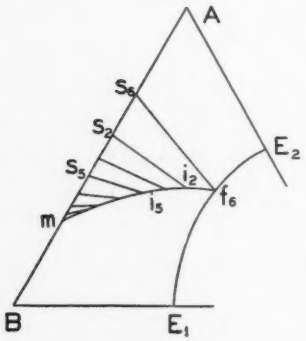

FIGURE 7

Tie lines joining the solid solutions s_2, s_5, etc., with the liquids i_2, i_5, etc., in equilibrium with them and on the curve mf_6 which corresponds with the curve mf_6 of figure 4.

If we consider x as moving on the line sy we see that an infinite number of equilibrium crystallization curves pass through y and according as x approaches y at the limit, the tangent to the equilibrium curve approaches the tangent to the fractionation curve at y. The curve of equilibrium crystallization yo of the liquid y and the curve of fractional crystallization Ayf through y thus have a common tangent at y. In terms of the physical process, this is true because there are initially no crystals present in the liquid y and consequently, even with perfect equilibrium crystallization, it has none of the component of motion away from B. However, as soon as a little crystallization has occurred the liquid acquires some component

of motion away from B and the equilibrium curve from y immediately departs from the fractionation curve yf, all points on it lying on that side of yf away from B. The equilibrium curve of the liquid y lies closer to the fractionation curve yf than any of the equilibrium curves that pass through y. As the point x approaches s as its limit the maximum departure of the portion of the equilibrium curve yz from the fractionation curve yf is experienced, because the maximum quantity of crystals is present and the maximum component of motion away from B is acquired. The actual

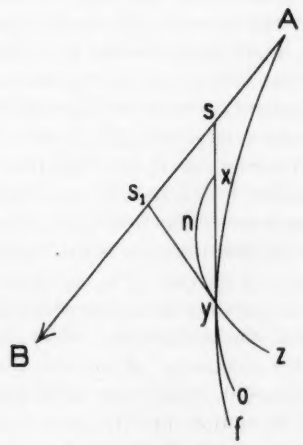

FIGURE 8

Showing the relations between the tangent sy to the fractionation curve through y and the tangent s_1y to an equilibrium curve through y.

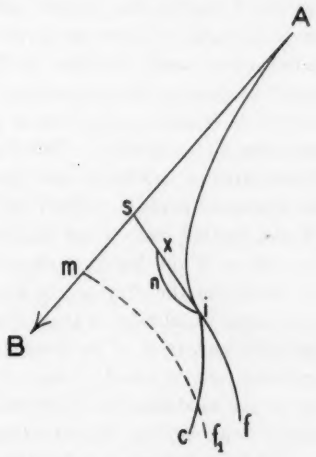

FIGURE 9

Showing the curve of equilibrium crystallization ($xnic$) with a corner point at i.

position of the equilibrium curve of a liquid approaching s is determined by specific properties of the system.

We may now apply these concepts to the special problem of the relations at an inflexion point on a fractionation curve. In figure 9 the curve Aif has an inflexion point at i and si is tangent to the curve at i. All tangents to the curve at points between A and i meet AB at points between s and A, and again all tangents to the curve at points between i and f meet AB at points between s and A. During fractional crystallization any liquid along Ai deposits mix-crystals with increasing content of B until a maximum content at s is attained, the liquid then having the composition i. Thereafter the content of B in the crystals decreases. With

perfect equilibrium crystallization, a liquid of composition *i* will follow a curve which initially has the same tangent *si* as the fractionation curve *if* but immediately thereafter departs from the curve *if* in such a manner that all points on it lie on the side of *if* away from *A*. The reason for this is that the crystals formed must have their composition changed from *s* towards *A* and this gives the liquid a component of motion away from *A* in addition to the component of motion along *if*. Any mixture of composition on the line *si*, such as *x*, will consist, at the temperature *i*, of the liquid *i* and mix-crystals *s*. The initial liquid *x* will have attained that condition by following the course *xni* which departs from the fractionation curve through *x* in virtue of the fact that the crystals are, during that period, continually enriched in *B* and the liquid therefore has the additional component of motion away from *B*. At *i* the liquid, in the absence of crystals, would tend to follow *if*, with formation of crystals of gradually increasing content of *A*. But there is present a large amount of crystals of composition *s* which, with perfect equilibrium, must also experience this enrichment in *A*. The liquid will therefore follow the curve *ic* which lies still farther away from the fractionation curve *if* than does the equilibrium curve of the liquid *i*, whose position has just been discussed, but is not shown on the diagram to avoid confusion of curves. The curve *xnic* experiences an abrupt change of direction at *i* for the reason that although the one component of motion, the one due to the fractionation effect, experiences only gradual change at *i*, the other component of motion, that due to the modification of crystals already present, experiences an abrupt change from motion directly away from *B* to motion directly away from *A*. The maximum angle between the tangents at *i* to the two segments of the curve *xnic* will be obtained when the point *x*, considered as moving on *is*, approaches *s* as its limit, and the minimum angle at *i* will be given by liquid approaching indefinitely close to *i*, for the magnitude of change of direction is naturally connected with the proportion of crystals present when *i* is reached.

In spite of the abrupt change of direction at *i* of the curve representing the changing composition of liquid during crystallization, the composition of the liquid experiences, of course, only continuous change. Likewise nothing happens of a drastic character to the crystals separating from the liquid. The point *i* represents simply that temperature and composition of the liquid at which the crystals separating during perfect equilibrium crystallization from any initial liquid on the line *si* have attained (like the crystals formed from all initial liquids on the curve *Aif* during perfect fractional crystallization) their maximum content of *B*. At temperatures below *i* the concentration of *B* in the crystals falls off. Attention is again directed to figure 7 which pictures the general arrangement of lines having the properties of *si*, there lettered s_2i_2, s_5i_5, etc.

Finally we may revert to the question raised earlier, that of the behavior of a curve of equilibrium crystallization when it crosses the fractionation curve which comes from the binary minimum. The answer is that it crosses that curve, as it does any other fractionation curve, without being affected in any way. The point of crossing is given by the tangent relation already discussed and developed with the aid of figure 5. To emphasize this fact we have shown in figure 9 the central fractionation curve mf_1 and the curve *xnic* crossing it, the point of intersection being determined by a tangent dropped from x upon mf_1 (not actually drawn).

If it should happen that the fractionation curve mf_1 were itself a reversed curve, then, of course, at one point only upon it, namely, its point of inflexion, curves of equilibrium crystallization would behave in the same manner as at any other inflexion point on a fractionation curve, and thus experience the abrupt change of direction and sign of curvature. In case the central fractionation curve has this inflected character, adjacent fractionation curves on both sides will have points of inflexion; indeed, on one side they will have two such points. The distribution of these special points will therefore be more complicated than in the case here chosen for discussion but the same principles will apply.

[1] Schreinemakers, F. A. H., *Zs. phys. Chem.*, **50**, 192 (1905).

[2] For a discussion of the concept of three-phase boundaries and their significance see N. L. Bowen, *Am. Jour. Sci.*, **38**, 222 (1914).

A GENERALIZATION OF THE EUCLIDEAN ALGORITHM TO SEVERAL DIMENSIONS

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Communicated May 9, 1941

If $Y = \Sigma a_i X_i$, where the a's are integers, we say that Y is an I.L.C. (integral linear combination) of the X's.

We say that a set of vectors $U_1, \ldots, U_r$ is a G.C.F. of a set of vectors $V_1, \ldots, V_s$ if:

(1) the U's are linearly independent,

(2) each U is an I.L.C. of the V's,

(3) each V is an I.L.C. of the U's.

A set of V's is said to be commensurable if the set of I.L.C.'s of the V's has no limit point.

It can be shown that every commensurable set of V's has a G.C.F.

For the one-dimensional case, the V's will be a set of collinear vectors.

If they are commensurable, their G.C.F. is a single vector, which can be found by the Euclidean algorithm. For instance, suppose one starts with two commensurable, collinear vectors, V_1 and V_2, V_2 shorter than V_1. Then one chooses an integer n which minimizes the length of $V_1 + nV_2$, and puts $V_3 = V_1 + nV_2$. Then V_3 is shorter than V_2, and one repeats the process with V_2 and V_3. And so on until one arrives at a G.C.F. of V_1 and V_2.

Now suppose that one has three commensurable, coplanar vectors, V_1, V_2 and V_3, of which V_1 is a longest and V_3 is a shortest. To find their G.C.F., let us try generalizing the procedure used for two collinear vectors. So choose integers m and n which minimize the lengths of $V_1 + mV_3$ and $V_2 + nV_3$, and put $V_4 = V_1 + mV_3$ and $V_5 = V_2 + nV_3$. In two dimensions, the minimum of $V_1 + mV_3$ for integral m may be V_1 itself; will be, in fact, if V_1 and V_3 are nearly perpendicular. However if V_1 is the minimum of $V_1 + mV_3$ for integral m and V_2 is the minimum of $V_2 + nV_3$ for integral n, then V_1 and V_2 are both nearly perpendicular to V_3, so that they are nearly collinear, so that $V_1 + mV_2$ can be made shorter than V_1 for properly chosen integral m, except for the special case (with its trivial variations due to reordering, etc.) where V_2 and V_3 have equal lengths and make an angle of 120° and $V_1 = V_2 + V_3$. In this case (which would be immediately recognized while looking for the minimum of $V_1 + mV_3$), V_2 and V_3 constitute a G.C.F. So one can find a G.C.F. of three commensurable, coplanar vectors.

For four commensurable, coplanar vectors, V_1, V_2, V_3, V_4, one first finds a G.C.F., U_1, U_2, of V_1, V_2, V_3, and then a G.C.F. of U_1, U_2, V_4. For still more vectors or still more dimensions the procedure is similar.

We inquire as to the computational aspect of performing this algorithm. Let (X, Y) denote the usual inner product of the vectors X and Y, so that $(X, X)^{1/2}$ is the length of X and

$$\frac{(X, Y)}{\sqrt{(X, X)}\,\sqrt{(Y, Y)}}$$

is the cosine of the angle between X and Y. Then the problem of minimizing the length of $Y + nX$ is just the problem of minimizing $(Y + nX, Y + nX)$. That is, we wish to minimize $(Y, Y) + 2n(X, Y) + n^2(X, X)$. Plot the parabola $y = (X, X)x^2 + 2(X, Y)x + (Y, Y)$. Clearly the lowest point on it is at $x = -(X, Y)/(X, X)$, and the lowest point with integral x is got by taking that integral value of x which is nearest to $-(X, Y)/(X, X)$.

By use of this algorithm, one can get much less awkward solutions of many types of Diophantine equations than by former methods. For example, we find the general solution in integers of

$$4296x + 6809y + 9975z = N$$

to be

$$\begin{aligned} x &= 71u + 90v + 54N \\ y &= -99u + 15v - 37N \\ z &= 37u - 49v + 2N \end{aligned}$$

Let A be a positive definite symmetric matrix and redefine the length of X, taking it to be $(X, AX)^{1/2}$. With appropriate changes, our earlier remarks about G.C.F.'s and generalized Euclidean algorithms still hold true. So (with slight additional refinements which have only been worked out for four or less dimensions) the algorithm will solve for positive definite quadratic forms, with matrix A, the problems:

1. To find the minimum of the form.
2. To find all automorphs of the form.
3. To tell if a second form is equivalent to the first, and, if it is, to find a transformation which carries one into the other.

The algorithm has been extremely successful in solving certain approximation problems, although the underlying theory has not been worked out in all cases. For instance, given real numbers $a_1, \ldots, a_n$, the algorithm furnishes sets of integers $x, x_1, \ldots, x_n$ such that simultaneously

$$\left| a_i - \frac{x_i}{x} \right| \leq \frac{1}{2} x^{-1-1/n}.$$

ON THE PROBLEM OF ANALYTICITY IN DYNAMICS

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The object of the following considerations is an explanation of a fairly general aspect of the geometrical dynamics of surface transformations, as inaugurated by Poincaré and further developed by Birkhoff.[1] The aspect in question may roughly be described by saying that the central results obtained, thus far, for the integrals or for the closed invariant sets of an incompressible flow are by no means sharper in case of an analytic flow than they are under the semi-topological assumption of mere differentiability (of a sufficiently high degree) or hypercontinuity. This situation leads to an alternative of fundamental methodical significance, the common aspect of the known results being due either to a mere inadequacy of the methods applied, thus far, in the analytic case or to the intrinsic nature of the problems involved.

Accordingly, the question is whether the limit process leading from the "general solution" to an "integral in the large" need or need not preserve the analytic character given with a flow. On the basis of a slightly simplified model, it will turn out in this note that the answer must be in the negative.

While from the point of view of the mathematician this decides only a methodical alternative, it appears to invalidate, even locally, certain efforts made by physicists in the direction of a general theory of conditionally periodic motions.[2] In fact, what will be shown is that such a motion can be regular analytic in the time if the associated multiply periodic function in the phase space not only fails to be regular but is not even absolutely continuous (though it is, of course, continuous). But in the latter case the gradient operations applied in the physical literature[3] are certainly illegitimate, and so the corresponding considerations become meaningless even from the local point of view.

The basic character of the tacit assumptions of the considerations just referred to was pointed out some time ago.[4] The counter-example to be constructed implies that the assumptions involved cannot be justified at all, at least not without specific restrictions depending on the canonical equations themselves.

By the elements of the theory of continued fractions, there exist for every irrational number λ two sequences of positive integers $m_1, m_2, \ldots$; $n_1, n_2, \ldots$ such that

$$|m_k - \lambda n_k| < 1/n_k \text{ for } k = 1, 2, \ldots \tag{1}$$

and

$$n_k \to \infty \text{ as } k \to \infty. \tag{2}$$

According to (2), the sequence $n_1, n_2, \ldots$ contains a subsequence $n_1', n_2', \ldots$ satisfying $n_k' > k^2$. Hence, if $m_1', m_2', \ldots$ denotes the corresponding subsequence of $m_1, m_2, \ldots$, then, on writing m_k and n_k for m_k' and n_k', one has, besides (1) and (2),

$$\sum_{k=1}^{\infty} \frac{1}{n_k} < \infty. \tag{3}$$

Consider the torus

$$0 \leqq x < 2\pi, \quad 0 \leqq y < 2\pi, \tag{4}$$

obtained from a real (x, y)-plane by reduction mod$(2\pi, 2\pi)$. According to (3),

$$F(x, y) = \sum_{k=1}^{\infty} \frac{1}{n_k} \cos (m_k x - n_k y) \tag{5}$$

defines a continuous function F of the position on the torus (4). In particular, if either y or x is fixed, (5) becomes a continuous periodic function of a single variable.

Neither of these functions is absolutely continuous. For otherwise they possessed Fourier series, and these could be obtained by formal differentiation of the series (5) with respect to x and y, respectively. But the resulting trigonometric series are seen to be

$$-\sum_{k=1}^{\infty} \frac{m_k}{n_k} \sin (m_k x - n_k y), \quad \sum_{k=1}^{\infty} \sin (m_k x - n_k y)$$

for F_x, F_y, respectively. And neither of these series is a Fourier series, since both violate the Riemann-Lebesgue lemma (the first because (1) and (2) imply that m_k/n_k tends to the non-vanishing limit λ, as $k \to \infty$).

Similarly, it is seen from the coefficients of the trigonometric series belonging formally to the second mixed derivative F_{xy}, that, in view of the two-dimensional analogue of the Riemann-Lebesgue lemma, the continuous function (5) of the position on the torus (4) cannot be an absolutely continuous function of the position (x, y).

Consider, however, the function $f(t)$, $-\infty < t < \infty$, to which (5) reduces along the characteristic

$$x = t - t_0, \quad y = \lambda t, \tag{6}$$

where t_0 is arbitrarily fixed. In other words, let

$$f(t) = F(t - t_0, \lambda t) \equiv \sum_{k=1}^{\infty} \frac{1}{n_k} \cos \{(m_k - \lambda n_k)t + \alpha_k\}, \tag{7}$$

where $\alpha_k = -m_k t_0$. This uniformly almost periodic function of the time turns out to be regular analytic along the whole t-axis. Furthermore, there exists in the plane of the complex variable $s = t + iu$ a transcendental entire function which is bounded in every strip parallel to the real axis and reduces to $f(t)$ for $u = 0$.

In fact, if $c > 0$ is arbitrary, the absolute value of $\cos (Z + \alpha) \equiv \cos (X + iY + \alpha)$ is, for real α, less than a constant multiple of $exp\ c$ in the strip $-c < Y < c$. Hence.

$$\cos \{(m_k - \lambda n_k)s + \alpha_k\}, \quad \text{where } s = t + iu, \tag{8}$$

is majorized by a constant multiple of $exp(|m_k - \lambda n_k|c)$ in the strip $-c < u < c$. But this exponential tends, by (1) and (2), to a finite limit as $k \to \infty$, if c is fixed. It follows, therefore, from (3) that, if $f(s) \equiv f(t + iu)$ is defined by writing s for t in the series (7), the resulting series is uniformly convergent and bounded in the strip $-c < u < c$. Since $c > 0$ can be chosen arbitrarily large, the proof is complete.

It will be observed that the passage from the rather non-analytic (though continuous) space function (5) to the regular time function (7) is based on a reversal of the rôle played by the Diophantine "small divisors" in Celestial Mechanics.[5] In fact, due to the lacunary property of the partial sums of (5), these small "integration divisors," which retard convergence, now appear replaced by small differentiation factors, which can only improve the convergence situation. For the jth derivative of (7) is either

$$f^{(j)}(t) = \pm \sum_{k=1}^{\infty} (m_k - \lambda n_k)^j \frac{\cos}{n_k} \text{ or } f^{(j)}(t) = \pm \sum_{k=1}^{\infty} (m_k - \lambda n_k)^j \frac{\sin}{n_k},$$

and (1) shows that even the formal convergence of these series is the more favorable the greater is j.

It seems to be likely that the relevant lacunary character cannot be realized in case of a regular Hamiltonian function. But this could depend only on specific formal facts concerning canonical differential equations, and not on mere function-theoretical facts expressible in terms of the flow alone.

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[1] Birkhoff, G. D., *Acta Math.*, **43**, 1–79 (1920), or *Ann. Inst. Poincaré*, **2**, 369–386 (1932).

[2] *Handbuch der Physik*, **5**, 133–136 (1927) and **9**, 179–182 (1926).

[3] References are given, loc. cit.[2]

[4] A. Wintner, *Amer. Jour. Math.*, **60**, 472 (1938).

[5] For references cf. A. Wintner, *The Analytical Foundations of Celestial Mechanics*, Princeton, 1941, pp. 442–443.

A GENERALIZATION OF FRÉCHET'S DIFFERENTIAL[1]

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In functional analysis it is Fréchet's well-known differential which has proved to be the most useful one. In the last few years several different definitions for differentials of the Fréchet type have been proposed for functions whose arguments and values lie in linear topological spaces.[2] The author believes that the definition given below has certain advantages over the others.

1. *F-differentials.*—Let L_1, L_2 be linear topological spaces and let D_1, D_2 be the associated "strongly partially ordered" spaces.[3] Let $|x|_d$ and and $|y|_e$, where $x \in L_1$, $d \in D_1$, $y \in L_2$, $e \in D_2$, be pseudo-norms[3] for L_1 and L_2, respectively.

Definition: The function $f(x)$ on an open set $G \subset L_1$ to L_2 will be called *F-differentiable* at the point x of G and $df(x; h)$ will be called its *F-differential* with increment h if the following conditions are satisfied:

(*a*) $df(x; h)$ is additive and continuous in h on L_1 to L_2;

(*b*) for every $e \in D_2$ there exists $d \in D_1$ such that, for every $\eta > 0$ there exists $\delta > 0$ such that $|h|_d < \delta$ implies that

$$|f(x + h) - f(x) - df(x; h)|_e \leqq \eta |h|_d.$$

Note that d depends only on e, while the number δ depends on e, d and η.

F-differentials have the usual elementary properties of differentials of the Fréchet type. In particular, all of the theorems stated by Michal for his *M*-differential in the 1938 paper referred to above[4] are also true for the *F*-differential. It can be shown that every *F*-differential is also an *M*-differential, but the converse is still undecided. The property of a function's being *F*-differentiable is invariant under topological isomorphisms of the spaces L_1 and L_2. Since a norm is a special case of a pseudo-norm, the definition of an *F*-differential is equivalent to that of Fréchet in case L_1 and L_2 are normed spaces. It is not yet known whether every *M*-differential is a Fréchet differential in the case of normed spaces. Even if it could be shown that *F*-differentials were the same as *M*-differentials, the present definition is direct and comparatively easy to apply, in the examples considered by the author.

2. *Differentiable Functions on the Space of Real Sequences.*—Denote the space of all real sequences by S. We may take the associated strongly partially ordered space D to be the set of positive integers with the usual ordering, and define[5] the pseudo-norm $|x|_d$ of $x = (x_1, x_2, \ldots)$ as max. $[|x_i|;\ 1 \leqq i \leqq d]$. The following theorem is easily proved.

THEOREM. *Let $f(x) = (f_1(x), f_2(x), \ldots)$ be a function on S to S such that each component $f_i(x)$ is a function of only a finite number of the real variables x_j, say $f_i(x) = f_i(x_{i_1}, \ldots, x_{i_r})$. Let $x^0 = (x_1^0, x_2^0, \ldots)$ be a point of the space S such that for each i the Stolz-Fréchet differential of $f_i(x_{i_1}, \ldots, x_{i_r})$ exists at $(x_{i_1}^0, \ldots, x_{i_r}^0)$. Then the function $f(x)$ has an F-differential at the point x^0.*

Michal has proposed[6] certain modifications of his M-differential in order to extend the definition to functions defined on topological abelian groups, and also to make the differential coincide with the Fréchet differential in the case of linear normed spaces. The important modification is the strong uniformity condition $2(d)$ (these PROCEEDINGS, **26,** 356 (1940)) which is to be satisfied by $\epsilon(x, h, k)$. We now show that these modified differentials do not coincide with the F-differential. Define $f(x) = (f_1(x), f_2(x), \ldots)$ on S to S by the equation $f_i(x) = x_i x_{i+1}$. Then $f(x)$ is F-differentiable at each point of S, by the above theorem. However it can be shown that no function $\epsilon(x, h, k)$ can be defined for $f(x)$ which satisfies the conditions $2(a)$, $2(b)$, and 3 of Michal[4] as well as the uniformity condition $2(d)$ referred to above. Therefore $f(x)$ is not differentiable in Michal's modified sense. Since every F-differential is an M-differential, the converse of Michal's theorem 5 (these PROCEEDINGS, **26,** 357 (1940)) is not true.

3. Differentiable Functionals.—The following theorem shows that the class of differentiable functionals is rather restricted.

THEOREM. *Let L be a linear topological space containing no bounded[7] open set and let $f(x)$ be a real valued functional defined on an open set G of L which is M-differentiable at a point x_0 of G. Then there exists a linear set M of L whose dimension is greater than zero and a linear functional $g(y)$ on M such that $f(x_0 + y) = g(y) + f(x_0)$ for all y in M.*

In the important special cases where L is an infinitely dimensional normed space with the weak topology and where L is the space S, the linear set M is infinitely dimensional.

[1] Presented in part to the American Mathematical Society on September 5, 1939. A more complete account will be published elsewhere.

[2] Michal, A. D., these PROCEEDINGS, **24,** 340–342 (1938) and **26,** 356–359 (1940). See also *Bull. Amer. Math. Soc.*, **45,** 529–563 (1939).

[3] Hyers, D. H., *Duke Math. Jour.*, **5,** 628–634 (1939). D_1 and D_2 may be thought of as neighborhood systems of the origin in L_1 and L_2.

[4] See the first paper mentioned in footnote 2.

[5] The topology defined by the pseudo-norm is equivalent to the topology defined by the Fréchet metric.

[6] Michal, A. D., these PROCEEDINGS, **26,** 356–359 (1940). See also a forthcoming paper by Michal in *Revista de Ciencias, Lima, Peru.*

[7] A set M is bounded (von Neumann's definition) if, for each neighborhood U of the origin, there exists a number α such that $M \subset \alpha U$.

A GENERALIZATION OF THE DIRAC EQUATIONS

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If $x_1, \ldots, x_4$ are rectangular coördinates in a Euclidean four dimensional space, the Dirac equations can be written

$$\frac{1}{\lambda}\sum_{m=1}^{4} A_m \frac{\partial \psi\cdot}{\partial x_m} - \psi\cdot = 0, \tag{1}$$

where $\lambda = 2\pi i/h$, $\psi\cdot = (\psi_{ij}) = (\psi_i)$ is a square matrix with four identical columns, and $A_m = (a_{ij}^{(m)})$, $m = 1, \ldots, 4$ are any particular four-by-four matrices such that

$$A_m A_n + A_n A_m = 2\delta_{mn} I. \tag{2}$$

It is known that if $\{A_m\}$ and $\{A'_m\}$ are any two sets of matrices satisfying (2), then there exists a matrix, C, unique except for a scalar factor, such that $A'_m = CA_mC^{-1}$ for each m. In this work we shall find it convenient to choose

$$\begin{pmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & -1 & 0 \\ 0 & 0 & 0 & -1 \end{pmatrix}, \begin{pmatrix} 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \\ 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \end{pmatrix}, \begin{pmatrix} 0 & 0 & i & 0 \\ 0 & 0 & 0 & -i \\ -i & 0 & 0 & 0 \\ 0 & i & 0 & 0 \end{pmatrix}, \begin{pmatrix} 0 & 0 & 0 & 1 \\ 0 & 0 & -1 & 0 \\ 0 & -1 & 0 & 0 \\ 1 & 0 & 0 & 0 \end{pmatrix} \tag{3}$$

as fixed values for A_1, A_2, A_3, A_4, respectively.

We now introduce a fifth matrix $A_0 = (a_{ij}^{(0)}) = iI$, which is equal to $-i$ times the coefficient of $\psi\cdot$ in (1), and let

$$A^* = (a_{ij}^*) = \sum_{m=0}^{4} A_m y_m,$$

where $y_0, \ldots, y_4$ form a set of independent variables. Then

(I). $|A^*| = Q^2 = (y_0^2 + y_1^2 + y_2^2 + y_3^2 + y_4^2)^2$,

(II). The cofactor of a_{ij}^* in $|A^*|$ is equal to $A_{ij}^* Q$, for some linear function A_{ij}^* of $y_0, \ldots, y_4$,

for all sets $\{A_m\}$ satisfying (2).[1]

It is easily verified that if $A_{ij}^* = \Sigma A_{ij}^{(m)} y_m$, then $A_{ij}^{(m)}$ is the cofactor of $a_{ij}^{(m)}$ in $|A_m|$, and

$$\left.\begin{aligned} A_{ij}^{(m)} &= a_{ji}^{(m)} \text{ for } m = 1, \ldots, 4, \text{ since } Am^{-1} = A_m, \\ A_{ji}^{(0)} &= -a_{ji}^{(0)}, \text{ since } A_0^{-1} = -A_0. \end{aligned}\right\} \tag{4}$$

We see that (I) is essentially a weaker assertion than (II), since (II) implies that $|A^*| = cQ^2$ for some constant c. In this note we shall use (I) and (II) as a starting point, and examine the equations which arise.

We begin by observing two groups of transformations which leave (I) and (II) invariant and which leave (I) invariant when (II) does not apply. First, if (C, D) is a pair of matrices such that $|C| \cdot |D| = 1$, and B^* satisfies (I) and (II), then so does CB^*D. For $|CB^*D| = |B^*|$, and each third order minor of $|CB^*D|$ is a linear combination of third order minors of $|B^*|$. Evidently we do not throw away any solutions by requiring that $|C| = |D| = 1$. If we then identify the pairs

$$(C, D),\ (iC, -iD),\ (-C, -D),\ (-iC, iD),$$

and define multiplication as

$$(C_1, D_1)\cdot(C_2, D_2) = (C_1C_2, D_2D_1),$$

the group of transformations is isomorphic to the group of pairs, which has 30 degrees of freedom. We shall use *pair* to denote either a pair of matrices or a transformation of the group.

Secondly, take any five-by-five orthogonal matrix $G = (g_{mn})$, $m, n = 0, \ldots, 4$, and let $y_m' = \Sigma_n g_{mn}y_n$. Let $B^* = \Sigma B_m y_m$ be any solution, and define $B'^* = \Sigma B_m' y_m$ by the identity $\Sigma B_m' y_m' = \Sigma B_m y_m$. Since G is orthogonal, $\Sigma y_m'^2 = \Sigma y_m^2$. Hence B'^* is a solution. We see that $B_m' = \Sigma_n g_{mn}B_n$, so that the set $\{B_m\}$ undergoes an orthogonal transformation. The group of such transformations, which we shall call *rotations*, has 10 degrees of freedom.

We now state that if B^* satisfies (I) and (II), then there exists a unique pair (C, D) such that $B^* = CA^*D$. The proof is roughly as follows: Since $|B_0| = 1$, (I, iB_0^{-1}) is a pair transforming B^* to a solution B'^* with $B_0' = A_0$. For any $m \neq 0$ we can find C so that $B_m'' = CB_m'C^{-1}$ is in diagonal or almost-diagonal form. Equating the coefficients of $y_0^3y_m$, $\ldots$, y_m^4 in $|B''^*|$ to the values required by (I), and solving, we see that the diagonal elements of B_m'' are those of A_1. The assumption that B_m'' is not diagonal leads to a contradiction of (II). Hence $CB_m'C^{-1} = A_1$, and $B_m'^2 = I$, for $m = 1, \ldots, 4$. Letting $B_0'' = A_0$, $B_m'' = A_1$, we see by similar reasoning that $B_m'B_n' + B_n'B_m' = 0$ for $n \neq 0$ or m. Hence $B_1', \ldots, B_4'$ satisfy (2), and the existence of a pair follows. The uniqueness is readily verified.

We have observed that for any rotation G, $A_m' = \Sigma g_{mn}A_n$ is a solution. It follows that to each rotation G there corresponds a unique pair (C_G, D_G) such that

$$\sum_{n=0}^{4} g_{mn}A_n = C_G A_m D_G,\ m = 0, \ldots, 4.^2 \tag{5}$$

If G leaves the axis of y_0 fixed, i.e., $g_{om} = g_{mo} = \delta_{mo}$, then $D_G = C_G^{-1}$.

We now remove condition (II) and show that (I) possesses more general solutions. In particular, we shall determine the most general matrix $R = (r_{ij}) = (r_i s_j)$ of rank $\leqq 1$, with elements of an unknown form, which may be added to A^* without changing the determinant. Since any two rows of R are proportional,

$$|A^* + R| - |A^*| = \sum_{i,j=1}^{4} r_i s_j A_{ij}^* Q = 0.$$

Using (4), we obtain the five equations

$$\sum_{i,j=1}^{4} r_i s_j a_{ji}^{(m)} = 0,\ m = 0, \ldots, 4. \tag{6}$$

The special values (3) of A_m show that equations (6) are consistent and determine s_j in terms of r_i to within a factor of proportionality.

$$s_1 = cr_2,\ s_2 = -cr_1,\ s_3 = cr_4,\ s_4 = -cr_3. \tag{7}$$

When R is a matrix of constants, we can choose r_i and s_j so that $c = 1$.

It is worth noting that R is also the most general matrix of rank $\leqq 1$ such that

$$RA_mR = 0 \text{ for } m = 0, \ldots, 4, \tag{8}$$

$$\text{i.e., } \sum_{j,k=1}^{4} r_i s_j a_{jk}^{(m)} r_k s_p = 0,\quad k, p = 1, \ldots, 4.$$

For unless each $r_i = 0$ or each $s_p = 0$, in which case $R = 0$, this reduces to (6). Any matrix $R = (r_i s_j)$ satisfying (6) will be called an *R-matrix.*

We wish solutions of (I) whose elements are linear homogeneous functions of the y_m. Taking any R-matrix R of constants and multiplying it by an arbitrary linear function $t^* = \sum_{m=0}^{4} t_m y_m$ gives a solution $A^* + t^*R$. The set of such solutions has 8 degrees of freedom.

Using the two groups of transformations, we can obtain further solutions. First, every pair (C, D) gives solutions

$$C(A^* + t^*R)D. \tag{9}$$

Secondly, every rotation G gives a solution B^* with

$$\begin{aligned} B_m &= \sum_n g_{mn}(A_n + t_n R) \\ &= C_G[A_m + (\sum_n g_{mn}t_n)(C_G^{-1}RD_G^{-1})]D_G. \end{aligned}$$

Since $C_G^{-1}RD_G^{-1}$ is of rank $\leqq 1$, it must be an R-matrix. Hence B^* is of the form (9). So the solutions (9) form a set closed under pairs and rotations. We shall say that under a rotation G, t_m transforms to $\sum_n g_{mn}t_n$, and R to $C_G^{-1}RD_G^{-1}$.

We can show that if $C(A^* + t^*R)D = C'(A^* + t'^*R')D'$, then $(C, D) = (C', D')$, and $t^*R = t'^*R'$. We first reduce the problem by a rotation to the case where $t_0 = t_1 = t_2 = 0$ and $t_0' = 0$. It is then tedious but not too difficult to verify the result. Thus the set of solutions (9) has 38 degrees of freedom.[3]

We have seen that any solution of (I) and (II) leads to the Dirac equations. Similarly, any solution B^* of (I) leads to a set of equations whose multiplier equation[4] is

$$y_1^2 + y_2^2 + y_3^2 + y_4^2 = 1,$$

being determined by (I). It is thus identical with the multiplier equation of (1). In particular, (9) yields the equations

$$\frac{1}{\lambda}\sum_{m=1}^{4}(A_m + t_mR)\frac{\partial\psi\cdot}{\partial x_m} - (I + t_0R)\psi\cdot = 0 \qquad (10)$$

When B^* is transformed by an operation leaving (I) invariant, the corresponding equations will be changed. First, a pair (C, D) leads to equations which are satisfied by $\psi'\cdot = D^{-1}\psi\cdot$ if $\psi\cdot$ satisfies the original equations. Hence a pair corresponds to a linear transformation of the dependent variables ψ_i. Secondly, a rotation G causes a transformation of the independent variables x_m, leaving fixed the quadric $x_1^2 + x_2^2 + x_3^2 + x_4^2 = 1$. This will be an orthogonal transformation of $x_1, \ldots, x_4$ if and only if G leaves the axis of y_0 fixed. In the following paragraphs we shall use *rotation* to refer only to those G mentioned above, whence $D_G = C_G^{-1}$.

Since equations (10) depend largely upon R, it is desirable to develop some more properties of R-matrices. Let $A_5 = A_1A_2A_3A_4$. Then for all G,

$$\begin{aligned} C_GA_5C_G^{-1} &= C_GA_1C_G^{-1} \ldots C_GA_4C_G^{-1} \\ &= \sum_{m_1, m_2, m_3, m_4} g_{1m_1}g_{2m_2}g_{3m_3}g_{4m_4}A_{m_1}A_{m_2}A_{m_3}A_{m_4}. \end{aligned}$$

Using (2) and the fact that G is orthogonal, we see that

$$C_GA_5C_G^{-1} = A_5 \text{ if } |G| = +1,$$

$$C_GA_5C_G^{-1} = -A_5 \text{ if } |G| = -1.$$

Now let $z_m = z_m(R) =$ trace of A_mA_5R, $m = 1, \ldots, 4$. Then if $R' = C_G^{-1}RC_G$,

$$\begin{aligned} z_m' = z_m(R') &= \text{trace of } A_m A_5 C_G^{-1} R C_G \\ &= \text{trace of } C_G A_m C_G^{-1} A_5 R \\ &= \text{trace of } \sum_n g_{mn} A_n A_5 R \\ &= \sum_n g_{mn} z_n, \end{aligned}$$

if $|G| = +1$. Thus $z(R) = (z_1, z_2, z_3, z_4)$ is actually a vector under rotations of determinant $+1$. If $|G| = -1$, $z(R)$ does not behave exactly like a vector, but instead $z_m' = -\sum_n g_{mn} z_n$. However, the set of all transformations on $z(R)$ is the same as the set of all rotations. Using the values (3) for A_m, and letting $c = 1$ in (7),

$$\begin{aligned} z_1 &= 2i(r_1 r_3 - r_2 r_4) \\ z_2 &= -i(r_1^2 - r_2^2 - r_3^2 + r_4^2) \\ z_3 &= -(r_1^2 + r_2^2 + r_3^2 + r_4^2) \\ z_4 &= 2i(r_1 r_2 + r_3 r_4). \end{aligned}$$

We see that $\sum_m z_m^2 = 0$. Hence there corresponds to each R-matrix a unique isotropic (or null) vector.

On the other hand, there is an infinity of R-matrices corresponding to each isotropic vector z. Consider first the case $z \neq 0$. Here there exists a rotation transforming z to $(0, -i, -1, 0)$. Solving,

$$r_1 = \cos\alpha, \quad r_2 = 0, \quad r_3 = 0, \quad r_4 = \sin\alpha, \text{ for some } \alpha, \qquad (11)$$

and the set of R-matrices corresponding to z has one degree of freedom. Explicit calculation reveals that if G is a rotation in the y_1-y_4 plane through -2α, then G transforms R, as given by (11), to an R-matrix with $r_1 = 1$, $r_2 = r_3 = r_4 = 0$. Hence all R with $z(R) \neq 0$ are equivalent under rotations. If G leaves R fixed, it obviously preserves $z(R)$. Since the set of all G preserving $z(R)$ has three degrees of freedom, the set of G preserving R must have two degrees of freedom. It follows that no unique vector independent of $z(R)$ can be associated with R. For the set of rotations preserving two linearly independent vectors has but one degree of freedom.

Similar methods show that the set of all $R \neq 0$ with $z(R) = 0$ has two degrees of freedom, and that all these R are equivalent under rotations, a normal form being $r_1 = 1, r_2 = 0, r_3 = 0, r_4 = i$. The set of G preserving R has four degrees of freedom; hence no unique non-null vector can be associated with R.

If we take the first term of (1), and substitute it for $\psi\cdot$ in (1), we build a second order equation, which reduces by (2) to

$$(\Box - I)\psi\cdot = 0, \tag{12}$$

where $\Box$ may be treated as a scalar-matrix operator with diagonal elements

$$\frac{1}{\lambda^2}\left(\frac{\partial^2}{\partial x_1{}^2} + \frac{\partial^2}{\partial x_2{}^2} + \frac{\partial^2}{\partial x_3{}^2} + \frac{\partial^2}{\partial x_4{}^2}\right)$$

Every solution of the Dirac equations must satisfy (12). Conversely, the Dirac equations have been obtained and studied as the most general system of four equations of first order, all of whose solutions satisfy (12).[5] Evidently $\Box$, and hence (12), are invariant under rotations.

If we use the same procedure with (10), we obtain a second order equation which will not reduce to a simple form. But if we continue and build a fourth order equation, it reduces by (2) and (8) to

$$[\Box - I][\Box - (I + t_0R)]\psi\cdot = 0. \tag{13}$$

Thus if $\psi\cdot$ satisfies (10), $[\Box - (I + t_0R)]\psi\cdot$ satisfies (12). Since $\Box$ is invariant under rotations, (13) is invariant under those rotations preserving R.

Thus the generalized equations (10) and the related equation (13), with $z(R) \neq 0$, might be suitable for a universe in which there is a preferred istropic direction, whereas the Dirac equations belong to a universe in which no direction is preferred. Such an isotropic direction may be identified with the possible path of a light ray or a photon.

Of particular interest is the case where $t_0 = 0$. Here the four dependent variables ψ_i are separated in (13), and each satisfies the same fourth order equation, which is invariant under all rotations. Of interest also may be the case where the vector (t_1, t_2, t_3, t_4) is a multiple of $z(R)$. For then any rotation which preserves R also preserves t^*, and equations (10) are themselves invariant under such rotations, in the sense that the Dirac equations are invariant. That is, under such rotations, the new solutions are linear combinations of the old ones. Finally, we mention that these two cases may be combined.

[1] Properties (I) and (II) were pointed out by Professor G. D. Birkhoff in lectures at Peiping (1934) as affording a possible starting point for a generalization of the Dirac equations.

[2] This fact is well known. The approach presented here merely affords an additional proof.

[3] At present I have been unable to determine whether (9) is the most general solution of (I).

[4] G. D. Birkhoff, "Quantum Mechanics and Asymptotic Series," *Bull. Amer. Math. Soc.*, **39**, 681–700 (1933).

[5] This approach was used by Birkhoff in the lectures at Peiping cited above.

GENERALIZATIONS OF COMPLETELY CONVEX FUNCTIONS

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A function $f(x)$ is said to be completely convex in an interval if it is real-valued and has derivatives of all orders (is of class C^∞) and if $(-1)^n f^{(2n)}(x) \geqq 0$ in that interval. D. V. Widder has shown that a completely convex function coincides in its interval of definition with an entire function of growth not exceeding order one and finite type.[1] One of the present authors has given a new proof of this theorem;[2] the other, a generalization.[3] In this note we give a much wider generalization, which also contains some results of S. Bernstein. This generalization was started by an (unpublished) note of the first named author, where a part of Theorem 2 (stated below) was proved, namely that $f(x)$ is entire and of finite order in case (I).

THEOREM 1. *Let $\{n_k\}$ and $\{q_k\}$ be sequences of positive integers, $\{n_k\}$ strictly increasing. Let $f(x)$ be real-valued and of class C^∞ in $-1 \leqq x \leqq 1$. For $k = 1, 2, \ldots$, let $f^{(n_k)}(x)$ and $f^{(n_k+2q_k)}(x)$ not change sign in $(-1, 1)$, and let*

$$f^{(n_k)}(x) f^{(n_k+2q_k)}(x) \leqq 0. \tag{1}$$

(I) If $n_k - n_{k-1} = O(1)$ and $q_k = O(1)$, then $f(x)$ coincides in $(-1, 1)$ with an entire function of growth not exceeding order one and finite type.

(II) If $n_k - n_{k-1} = O(n_k^\delta)$, $q_k = O(n_k^\delta)$, and $q_1 + q_2 + \ldots + q_k = O(n_k)$, where δ is fixed, $0 < \delta < 1$, then $f(x)$ coincides in $(-1, 1)$ with an entire function of finite order not exceeding $1/(1-\delta)$.

(III) If $n_k - n_{k-1} = o(n_k)$, $q_k = o(n_k)$, and $q_1 + q_2 + \ldots + q_k = O(n_k)$, then $f(x)$ coincides in $(-1, 1)$ with an entire function.

This theorem contains (for $q_k = 1$) certain results of S. Bernstein,[4] who derived them on the more restrictive hypothesis that no derivative of $f(x)$ changes sign in $(-1, 1)$. An interesting special case (where $2q_k = n_{k+1} - n_k$) is the following, a direct generalization of Widder's result.

THEOREM 2. *Let $\{n_k\}$ be a strictly increasing sequence of positive even integers. Let $f(x)$ be real-valued and of class C^∞ in $(-1, 1)$, and let*

$$(-1)^k f^{(n_k)}(x) \geqq 0 \qquad (k = 1, 2, \ldots). \tag{2}$$

(I) If $n_k - n_{k-1} = O(1)$, $f(x)$ coincides in $(-1, 1)$ with an entire function of growth not exceeding order one and finite type.

(II) If $n_k - n_{k-1} = O(n_k^\delta)$, $0 < \delta < 1$, $f(x)$ coincides in $(-1, 1)$ with an entire function of finite order not exceeding $1/(1-\delta)$.

(III) *If* $n_k - n_{k-1} = o(n_k)$, $f(x)$ *coincides in* $(-1, 1)$ *with an entire function.*

THEOREM 3. *The results stated in Theorems* 1 *and* 2 *are "best" results in the following sense:*

(I, II) *Corresponding to a given* δ, $0 < \delta < 1$, *we can construct an increasing sequence* $\{n_k\}$ *of even integers and an entire function* $f(x)$ *such that* (2) *is satisfied,* $(n_k - n_{k-1})n_k^{-\delta}$ *tends to a positive limit, and* $f(x)$ *is exactly of order* $1/(1-\delta)$.

(III) *Corresponding to a given positive* ϵ *we can construct an increasing sequence* $\{n_k\}$ *of even integers and a function* $f(x)$, *analytic in* $(-1, 1)$, *such that* (2) *is satisfied,* $(n_k - n_{k-1})/n_k$ *tends to* ϵ, *but* $f(x)$ *is not entire.*

Our proof of Theorem 1, which we give in outline, depends on the following lemma.

LEMMA. *If, in* $-1 \leqq x \leqq 1$, $g(x)$ *is real-valued, of class* C^{p+2q}, *and satisfies*

$$|g(x)| \leqq M, \quad g^{(p+2q)}(x) \leqq 0,$$

then

$$g^{(p)}(x) \leqq A^{p+2q}(p+2q)^p M$$

in $(-1, 1)$, A *being an absolute constant (independent of the positive integers* p *and* q *and of the function* $g(x)$).

By Taylor's theorem with remainder,

$$g^{(p)}(x) = g^{(p)}(0) + \frac{xg^{(p+1)}(0)}{1!} + \ldots + \frac{x^{2q-1}g^{(p+2q-1)}(0)}{(2q-1)!} + \frac{x^{2q}g^{(p+2q)}(\xi)}{(2q)!},$$

where $-1 < \xi < 1$ since $-1 \leqq x \leqq 1$. By hypothesis, the remainder is not positive and $g^{(p+2q-1)}(x)$ is monotonic. Therefore,[5] in $-1 + h \leqq x \leqq 1 - h$, where $0 < h < 1$,

$$|g^{(p+2q-1)}(x)| \leqq (p+2q-1)!\,\frac{1}{2}\left(\frac{4}{h}\right)^{p+2q-1} M.$$

Hence we deduce, by a theorem of A. Gorny,[6] inequalities for $|g^{(\nu)}(0)|$, $\nu = 1, 2, \ldots, p+2q-2$; from these, the lemma follows.

To prove Theorem 1, denote the maximum of $|f^{(n)}(x)|$ in $(-1, 1)$ by M_n. We apply the lemma to $g(x) = \pm f^{n_k-1}(x)$, with $p = n_k - n_{k-1}$ and $q = q_k$. We obtain

$$M_{n_k} \leqq A^{n_k - n_{k-1} + 2q_k}(n_k - n_{k-1} + 2q_k)^{n_k - n_{k-1}} M_{n_{k-1}}.$$

Discussion of this recursive inequality gives appropriate upper bounds for $M_{n_2}, M_{n_3}, \ldots$. From these we pass, by the same theorem of Gorny, to an

appropriate upper bound for $|f^{(n)}(x)|$ in $-1+\epsilon \leqq x \leqq 1-\epsilon$, $n = 0, 1, 2, \ldots$.

[1] Widder, D. V., "Functions Whose Even Derivatives Have a Prescribed Sign," these PROCEEDINGS, **26**, 657–659 (1940).

[2] Boas, R. P., Jr., "A Note on Functions of Exponential Type," forthcoming in *Bull. Am. Math. Soc.*

[3] Pólya, G., "On Functions Whose Derivatives Do Not Vanish in a Given Interval," these PROCEEDINGS, **27**, 216–218 (1941).

[4] Bernstein, S., "On Certain Properties of Regularly Monotonic Functions," [in Russian], *Comm. Soc. Math. Kharkow*, (4) **2**, 1–11 (1928).

[5] Landau, E., "Über einen Satz von Herrn Esclangon," *Math. Annalen*, **102**, 177–188 (1929); S. Bernstein, *Leçons sur les propriétés extrémales* . . ., Paris, 1926, p. 10.

[6] Gorny, A., "Contribution à l'Étude des Fonctions Dérivables d'Une Variable Réelle," *Acta Math.*, **71**, 317–358 (1939).

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*NATURAL SYSTEMS: THE STRUCTURE OF ABSTRACT MONOTONE SEQUENCES**

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1. In a previous communication[1] the author introduced the problem: to characterize the possible regular orderings of a natural system. A complete characterization was there given for the simplest non-trivial case, the natural system N_2 (containing exactly 2 primes). In the present paper a statement of the solution of this problem for the general case N_k (k primes, $1 \leqslant k \leqslant \aleph_0$)[2] and also that of the principal lemma are given, without proofs.

2. A natural system (with unit)[3] is an abstract system $(N, \circ)$ with single composition $\circ$ (called simply "product") satisfying (1°)–(7°) (in which, as elsewhere, $\sigma\circ\tau$ is abbreviated by $\sigma\tau$). For all elements σ, τ, φ of the class N

(1°) $\sigma\tau$ is a unique element of N.
(2°) $\sigma(\tau\varphi) = (\sigma\tau)\varphi$.
(3°) $\sigma\tau = \tau\sigma$.
(4°) N contains a unique unit element ϵ:$(\epsilon\,\sigma = \sigma\,\epsilon = \sigma)$.
(5°) N contains at least one prime (= irreducible) element $\alpha \neq \epsilon$:$(\alpha = \varphi\psi$ implies $\varphi = \epsilon$ or $\psi = \epsilon)$.
(6°) Each element $\neq\epsilon$ of N can be expressed as the product of (a finite number of) prime elements in exactly one way.
(7°) N is a denumerably infinite class.

The abstractly distinct natural systems may be listed as $N_1, N_2, \ldots,$

$N_k, \ldots, N_{\aleph_0}$, where N_k is *the* natural system containing exactly k primes, which latter we always denote by $\alpha_1, \alpha_2, \ldots, \alpha_k$.

An infinite sequence $\lambda, \mu, \ldots$ of elements of a natural system $(N_k, \circ)$ in which each element of N_k appears exactly once is called a *regular ordering* of $(N_k, \circ)$ if for all σ, τ, λ of N_k

$$\sigma :< \tau \text{ implies } \gamma\sigma :< \gamma\tau$$

where $:<$ is an abbreviation for "precedes." As we are only interested in abstractly different regular orderings of $(N_k, \circ)$, i.e., such as cannot be made identical by merely relettering their primes, we agree in the case of any regular ordering of $(N_k, \circ)$ to denote the first, second, ..., prime by α_1, $\alpha_2, \ldots, \alpha_k$, respectively. It is easily seen that in any regular ordering of $(N_k, \circ)$ the first element must be the unit ϵ and the second element the prime α_1.

3. We once and for all interchangeably identify the elements of N_k with the ("non-negative") lattice points (also regarded as central lattice vectors) of k dimensional real space R_k by means of the correspondence

$$\alpha_1^{m_1}, \alpha_2^{m_2}, \ldots, \alpha_k^{m_k} \longleftrightarrow (m_1, m_2, \ldots, m_k).$$

Also "line" always means: straight line through the origin. The projection of the lattice point P on the line Γ is denoted by $\Gamma(P)$. Further $\sigma \equiv \tau(\Gamma)$ (read: σ congruent τ mod Γ) denotes that $\Gamma(\sigma) = \Gamma(\tau)$. Finally if $\sigma = \alpha_1^{m_1}, \alpha_2^{m_2}, \ldots, \alpha_k^{m_k}$, $\tau = \alpha_1^{m_1'}, \alpha_2^{m_2'}, \ldots, \alpha_k^{m_k'}$, then by $\sigma\tau^{-1}$ is meant the lattice point $(m_1 - m_1', m_2 - m_2', \ldots, m_k - m_k')$ of R_k.

The line Γ of R_t with direction, $\mathfrak{v}_1, \mathfrak{v}_2, \ldots, \mathfrak{v}_t$ ($\mathfrak{v}_i$ real) is of *rank* r in R_t if some subset of $t - r$ of the $\mathfrak{v}_i$ but no subset of $t - r + 1$ of them are linearly independent (with rational coefficients). This is equivalent to saying: the set $\{X\}$ of all central lattice vectors X of R_t which are orthogonal to Γ contains a linear base $A_1, A_2, \ldots, A_r$ of r lattice vectors but no such of $r - 1$ vectors. The R_r (real space of r dimensions) spanning $A_1, A_2, \ldots, A_r$ depends then only on R_t, on the line Γ in R_t, and on the rank r of Γ in R_t; this R_r we denote by $R(\Gamma^{(r)}, R_t)$. Here the integer r satisfies $0 \leqslant r \leqslant k - 1$, and $r = 0$ means: $R(\Gamma^{(0)}, R_t)$ consists merely of the origin.

4. A finite sequence of mutually orthogonal lines $\Gamma_1, \Gamma_2, \ldots, \Gamma_g$ of R_k each of which has been assigned a sense is called a *g-frame* $\mathfrak{S}_{k,g} = (\overrightarrow{k}\ \Gamma_1, \Gamma_2, \ldots, \Gamma_g)$. Two such $(\overrightarrow{k}\ \Gamma_1, \ldots, \Gamma_g)$ and $(\overrightarrow{k}\ \Gamma_1', \Gamma_2', \ldots, \Gamma_{g'}')$ are *identical* only if $g = g'$, $\Gamma_i = \Gamma_i'$ and sense of Γ_i = sense of Γ_i' ($i = 1, 2, \ldots, g$); otherwise they are *different*.

A g-frame $(\overrightarrow{k}\ \Gamma_1, \ldots, \Gamma_g)$ is called a *canonical* g-frame if (8°) and (9°) are satisfied.

$$(8^\circ)\quad \begin{cases} \Gamma_1 \text{ is in } R_{r_0}(= R_k). \quad \text{Let } r_1 = \text{rank of } \Gamma_1 \text{ in } R_{r_0}. \\ \Gamma_2 \text{ is in } R_{r_1}(= R(\Gamma_1^{(R_1)}, R_{r_0})). \quad \text{Let } r_2 \text{ be the rank of } \Gamma_2 \text{ in } R_{r_1}. \\ \vdots \\ \Gamma_i \text{ is in } R_{r_{i-1}}(= R(\Gamma_{i-1}^{(R_{i-1})}, R_{r_{i-2}})). \quad \text{Let } r_i \text{ be the rank of } \Gamma_i \text{ in } R_{r_{i-1}}. \\ \vdots \end{cases}$$

The r_i of (8°) clearly satisfy $r_1 > r_2 > \ldots > r_g \geqq 0$.

(9°) $r_g = 0$.

By the *projection ordering* $N_k : \mathfrak{S}_{k,g}$ of the natural system N_k on the g-frame $\mathfrak{S}_{k,g}$ is meant the class N_k with its elements ordered according to their respective projections on the sensed line Γ_1 of $\mathfrak{S}_{k,g}$; elements (if any) not thereby ordered, i.e., congruent elements mod Γ_1, are ordered according to their respective projections on the sensed line Γ_2 of $\mathfrak{S}_{k,g}$; any elements still not thereby ordered are ordered according to their respective projections on the sensed line Γ_3 of $\mathfrak{S}_{k,g}$; etc., etc.

We can now state the

PRINCIPAL THEOREM ON THE STRUCTURE OF REGULAR ORDERINGS. *Each regular ordering Θ of N_k is identical with the projection ordering $N_k : \mathfrak{S}_{k,g}$ of N_k on one and only one canonical g-frame $\mathfrak{S}_{k,g}$.*

The proof depends mainly on the

PRINCIPAL LEMMA. *Let $\sigma_1 :< \tau_1$, $\sigma_2 :< \tau_2$, $\ldots$, $\sigma_t :< \tau_t$ be satisfied in a given regular ordering Θ of N_k, in which the central lattice vectors $S_1, S_2, \ldots, S_t$ (where $S_i = \sigma_i \tau_i^{-1}$) of R_k are linearly independent (with rational coefficients). Then in the real space R_t spanned by $S_1, \ldots, S_t$ there exists one and only one sensed line Γ_1 such that for all lattice points $\xi\eta^{-1}$ of $R_t : \xi :< \eta$ in Θ whenever $\Gamma(\xi)$ precedes $\Gamma(\eta)$.*

The geometrical formulation of the Principal Theorem can of course easily be translated into one involving only sequences of linear forms.

* Presented to the American Math. Society, Dec. 2, 1939.

[1] "Natural Systems," these PROCEEDINGS, **24**, No. 4, 185–187 (1938).

[2] The Principal Theorem is true also for $k = \aleph_0$ but the proof for this case requires an argument not necessary for k finite in view of the fact that a residue class of N_k mod γ may contain an infinite number of elements.

[3] In the previous communication referred to above it was thought desirable to define the concept natural system to expressly exclude a unit rather than to expressly demand one, as here. This revision, of trivial mathematical significance, results in a much more manageable formulation.

THE METHOD OF THE MINIMUM INTEGRAL AND ANALYTIC CONTINUATION OF FUNCTIONS OF COMPLEX VARIABLES

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1. The method of the minimum integral. The mapping by a pair of a.f. of 2 c.v. (analytic functions of two complex variables), PT (pseudo-conformal transformation), is a natural generalization of the concept of CT (conformal transformation). However, it has not been possible to find a suitable generalization of the lemma of Schwarz-Pick for PT's. The METHOD OF THE MINIMUM INTEGRAL is a substitute of this lemma. In this procedure, the Euclidean and non-Euclidean measures of geometrical objects (e.g., the volume of a domain or the area of a surface) are expressed in terms of minima λ of ${}^1\int_{\mathfrak{B}}|h|^2\,d\omega$. Here h runs through all functions regular in the domain $\mathfrak{B}$ which satisfy certain conditions at the point $(t) \epsilon \mathfrak{B}$, $(t) = (t_1, t_2)$. E.g., if these conditions are $h(t) = 1$, the resulting $\lambda \equiv \lambda^1_{\mathfrak{B}}(t)$ is equal to $[K_{\mathfrak{B}}(t, \bar{t})]^{-1}$ where $K_{\mathfrak{B}}(t, \bar{t}) = \sum_{\nu=1}^{\infty} |\varphi_\nu(t)|^2$. $\{\varphi_\nu(z)\}$ is a system of orthonormal a.f. of 2 c.v. z_1, z_2, complete for the class $E(\mathfrak{B})$ of a.f. of 2 c.v., f, which are regular in $\mathfrak{B}$ and have the properties that[2] $\int_{\mathfrak{B}}|f|^2 d\omega < \infty$. *If* $(t) \epsilon \mathfrak{G} \subset \mathfrak{S}$, *then*

$$\lambda^1_{\mathfrak{G}}(t) \leqq \lambda^1_{\mathfrak{S}}(t), \tag{1.1}$$

and therefore

$$K_{\mathfrak{G}}(t, \bar{t}) \geqq K_{\mathfrak{S}}(t, \bar{t}) \tag{1.2}$$

(see [1],[1] p. 45). Since, in the case of CT's the quantity $ds^2_{\mathfrak{B}^1}(z) = K_{\mathfrak{B}^1}(z, \bar{z})|dz|^2$ defines a Hermitian metric invariant with respect to CT's (see [1],[1] pp. 53 and 56), the lemma of Schwarz-Pick (in a slightly weaker form) follows from (1.2). That is, we have the following

LEMMA. *If the* CT w *given by* $w = w(z)$ *transforms* $\mathfrak{B}^2$ *into* $\mathfrak{G}^2 \subset \mathfrak{S}^2$, *then*

$$ds^2_{\mathfrak{B}^2}(z) \equiv K_{\mathfrak{B}^2}(z, \bar{z})|dz|^2 = K_{\mathfrak{G}^2}(w, \bar{w})|dw|^2 \geqq K_{\mathfrak{S}^2}(w, \bar{w})|dw|^2 \equiv ds^2_{\mathfrak{S}^2}(w) \tag{1.3}$$

2. B-area. Let $\mathfrak{L}^2 = E[z_k = z_k(u_1, u_2), 0 \leqq u_k \leqq 1, k = 1, 2]$ be a segment of a surface, $\mathfrak{L}^2 \subset \mathfrak{B}$. The quantities

$$B(\mathfrak{L}^2) = \int_0^1\int_0^1 \left|\frac{D(z_1, z_2)}{D(u_1, u_2)}\right| du_1 du_2,$$

$$B_{\mathfrak{B}}(\mathfrak{L}^2) = \int_0^1\int_0^1 \sqrt{K_{\mathfrak{B}}(z, \bar{z})}\left|\frac{D(z_1, z_2)}{D(u_1, u_2)}\right| du_1 du_2 \tag{2.1}$$

are called the B-area and the non-Euclidian B-area, respectively, of $\mathfrak{L}^2$ (see [3],[2] p. 476). The introduction of the B-area enables us to generalize (1.3) in the following manner: *Let the* PT W, *given by* $w_k = w_k(z_1, z_2)$, $k = 1,2$, *transform* $\mathfrak{B}$ *into a domain* $\mathfrak{G} \subset \mathfrak{S}$. *Let* $d\Omega_{\mathfrak{B}}(z_1, z_2)$ *and* $d\Omega_{\mathfrak{S}}(w_1, w_2)$ *be the non-Euclidean* B-*area of the element of the surfaces* $\mathfrak{L}^2$ *and* $W(\mathfrak{L}^2)$, *respectively. Then the inequality*

$$d\Omega_{\mathfrak{B}}(z_1, z_2) \equiv \sqrt{K_{\mathfrak{B}}(z, \bar{z})}\left|\frac{D(z_1, z_2)}{D(u_1, u_2)}\right| du_1 du_2 =$$

$$\sqrt{K_{\mathfrak{G}}(w, \bar{w})}\left|\frac{D(w_1, w_2)}{D(u_1, u_2)}\right| du_1 du_2 \geqq \sqrt{K_{\mathfrak{S}}(w, \bar{w})}\left|\frac{D(w_1, w_2)}{D(u_1, u_2)}\right| du_1 du_2 \equiv$$

$$d\Omega_{\mathfrak{S}}(w_1, w_2) \quad (2.2)$$

is valid.

3. Refinements of the inequalities (1.2) **and** (2.2). The inequalities (1.2) and (2.2) can be improved if the complex moments with certain weighting functions[3] of the domain $\mathfrak{G}^2 \times \mathfrak{G}^2$, $\mathfrak{G}^2 = W(\mathfrak{B}^2)$ are known.

LEMMA. *Let* $\mathfrak{G}^2 \subset \mathfrak{S}^2$. *The minimum* $\lambda^{[n]}_{\mathfrak{S}^2\mathfrak{G}^2}$ *of* $\int_{\mathfrak{S}^2} |h|^2 d\omega$ *under the conditions*

$$h(t) = 1, \quad \int_{\mathfrak{G}^2} z^\nu \bar{h} d\omega = 0, \quad \nu = 0, 1, 2, \ldots, n-2, \qquad (3.1)$$

is given by

$$\lambda^{[n]}_{\mathfrak{S}^2\mathfrak{G}^2}(t) = \frac{\begin{vmatrix} F_{11} & \cdots & F_{1n-1} \\ \cdots & & \cdot \\ F_{n-11} & \cdots & F_{n-1n-1} \end{vmatrix}}{\begin{vmatrix} K(t,\bar{t}) & \int K(t,\bar{\zeta})d\omega_\zeta & \cdots & \int \zeta^{n-2} K(t,\bar{\zeta})d\omega_\zeta \\ \int K(z,\bar{t})d\omega_z & F_{11} & \cdots & F_{1n-1} \\ \cdot & \cdot & \cdots & \cdot \\ \int \bar{z}^{n-2} K(z,\bar{t})d\omega_z & F_{n-11} & \cdots & F_{n-1n-1} \end{vmatrix}}, \qquad (3.2)$$

$$F_{pq} = \int\int \bar{z}^{p-1}\zeta^{q-1} K(z,\bar{\zeta})d\omega_\zeta d\omega_z, \quad K \equiv K_{\mathfrak{G}^2}, \quad \int \equiv \int_{\mathfrak{G}^2}.$$

In the case of PT's we obtain an analogous formula.

Proof. Let $\{\varphi_\nu(z)\}$ be a system of orthonormal functions in $\mathfrak{S}^2$, complete for $E(\mathfrak{S}^2)$. Every function $f \in E(\mathfrak{S}^2)$ can then be written in the form[5] $\Sigma A_\nu \varphi_\nu(z)$. The desired minimum is equal to the minimum of $\Sigma|A_\nu|^2$ under the conditions $\Sigma A_\nu \varphi_\nu(t) = 1$ and $\Sigma A_\nu \alpha_{q\nu} = 0$, $q = 1, 2, 3, \ldots, n-1$, where $\alpha_{q\nu} = \int_{\mathfrak{G}^2} z^{q-1}\overline{\varphi_\nu(z)}d\omega$. By the formula (4), p. 44 of [1][1] we obtain (3.2).[6]

Suppose now that $\{\varphi_\nu(z)\}$ forms a system of orthonormal functions in $\mathfrak{G}^2$ complete for $E(\mathfrak{G}^2)$. Suppose further that the first $(n-1)$ functions are polynomials obtained by the orthogonalization of $1, z, \ldots z^{n-2}$, and of $1, z_1, z_2, z_1^2, z_1z_2, z_2^2, \ldots$ in the case of two variables. Since the conditions

$\int_{\mathfrak{G}^2} z^\nu \bar{h} d\omega = 0$, $\nu = 0, 1, \ldots n-2$, are equivalent to $\int_{\mathfrak{G}^2} \varphi_\nu \bar{h} d\omega = 0$, $\nu = 1, 2, \ldots n-1$, we obtain $\sum_{\nu=n}^{\infty} |\varphi_\nu(z)|^2 = [\lambda^{[n]}_{\mathfrak{G}^2\mathfrak{G}^2}(z)]^{-1}$. Since, furthermore, $\lambda^{[n]}_{\mathfrak{S}^2\mathfrak{G}^2}(z) \leqq \lambda^{[n]}_{\mathfrak{S}^2\mathfrak{G}^2}(z)$, (see [1],[1] p. 45) we obtain the inequality[4]

$$K_{\mathfrak{G}^2}(z, \bar{z}) = \sum_{\nu=1}^{n-1} |\varphi_\nu(z)|^2 + [\lambda^{[n]}_{\mathfrak{S}^2\mathfrak{G}^2}(z)]^{-1} \geqq \sum_{\nu=1}^{n-1} |\varphi_\nu(z)|^2 + [\lambda^{[n]}_{\mathfrak{S}^2\mathfrak{G}^2}(z)]^{-1}. \quad (3.3)$$

By (2.1), (2.2) and (3.3) (for $n = 2$) generalized for the case of PT it follows

$$\mathbf{B}_{\mathfrak{B}}(\mathfrak{P}^2) \geqq \frac{1}{\sqrt{2}} \left\{ \frac{\mathrm{B}[\boldsymbol{W}(\mathfrak{P}^2)]}{\sqrt{\mathrm{Vol.}[\boldsymbol{W}(\mathfrak{B})]}} + \int_{\boldsymbol{W}(\mathfrak{P}^2)} \left| \frac{D(w_1, w_2)}{D(u_1, u_2)} \right| \times \right.$$
$$\left. [\lambda_{\mathfrak{B}, \boldsymbol{W}(\mathfrak{B})}^{[2]}(w_1, w_2)]^{-1/2} du_1 du_2 \right\} \quad (3.4)$$

4. A method for analytical extension of a.f. of 2 c.v. Let the CT, $\boldsymbol{w}$, given by $w = w(z)$ map $\mathfrak{C}^2 = \mathrm{E}[|z| < 1]$ into a domain $\mathfrak{G}^2$, $\mathfrak{G}^2 < \mathfrak{C}^2$, and let $\boldsymbol{w}$ transform a segment $\mathfrak{f}^1$ of the boundary of $\mathfrak{C}^2$ into an arc of a circle. That is, let $\boldsymbol{w}(\mathfrak{C}^2) = \mathfrak{G}^2$ and let $\boldsymbol{w}(\mathfrak{f}^1) = \mathrm{E}[|w - c| = C]$ where c and C are appropriate constants. It follows, then, by the method of reflection that $w(z)$ is regular in $\mathfrak{S}^2 = \mathfrak{C}^2 + \mathfrak{f}^1 + \mathfrak{M}[\mathfrak{f}^1, \mathfrak{C}^2]$, $\mathfrak{M}[\mathfrak{f}^1, \mathfrak{C}^2]$ being the domain obtained by the reflection of $\mathfrak{C}^2$ on $\mathfrak{f}^1$. Thus, every segment $\mathfrak{f}^{*1}$, $\overline{\mathfrak{f}^{*1}} \subset \mathfrak{f}^1$, lies in the interior of $\mathfrak{S}^2$. We may now apply the principle of non-increase of the hyperbolic measure or the method of the minimum integral in order to obtain bounds for the length of $\boldsymbol{w}(\mathfrak{f}^{*1})$.

In order to proceed analogously in the case of PT's, we shall develop a method for analytical continuation of a.f. of 2 c.v. This method is based on an integral formula given in [5],[7] where all new notations and symbols used below are explained.

First of all we shall introduce the concept of a REGULAR INTERSECTION OF A SEGMENT $\mathfrak{a}^3$ OF THE BOUNDARY $\mathfrak{b}^3$ OF $\mathfrak{B}$ WITH A SEGMENT OF AN A.H.-S. (analytical hypersurface) $\mathfrak{h}^3$, $\mathfrak{h}^3 = \mathrm{E}[\Phi(z_1, z_2, \lambda) = 0] = \mathrm{E}[z_k = h^k(\mathrm{Z}, \lambda), k = 1, 2] = \sum_{\lambda \in \mathfrak{f}^1} \mathfrak{H}^2(\lambda)$. (See p. 853 of [5].[7]) We suppose, however, that $h^k(\mathrm{Z}, \lambda)$ are a.f. of 1 c.v. Z regular in $\boldsymbol{R}_\lambda^{-1}[\mathfrak{H}^2(\lambda)]$. Further we suppose that:

(1) $\mathfrak{H}^2(\lambda) = \mathfrak{H}^2(\lambda')$ for $\lambda' = \lambda + 2k\pi$, k integer.

(2) Every lamina $\mathfrak{H}^2(\lambda)$, $\lambda \in \mathfrak{f}^1$, is decomposed by $\mathfrak{k}^1(\lambda) = \mathfrak{a}^3 \cdot \mathfrak{H}^2(\lambda)$ into two parts $\mathfrak{W}^2(\lambda)$ and $\mathfrak{Z}^2(\lambda)$, $\mathfrak{B} \cdot \mathfrak{Z}^2(\lambda) = 0$, $\mathfrak{W}^2(\lambda) \subset \mathfrak{B}$.

(3) $\boldsymbol{R}_\lambda^{-1}[\mathfrak{k}^1(\lambda)]$ is for every λ an analytic curve in the Z-plane.

(4) $\mathfrak{k}^1(\lambda)$ varies continuously with λ.

(5) There exists a simply connected part $\mathfrak{W}^{*2}(\lambda)$ of $\mathfrak{W}^2(\lambda)$ to the boun-

dary of which $\mathfrak{k}^1(\lambda)$ or a segment of $\mathfrak{k}^1(\lambda)$ belongs, such that $\mathfrak{W}^{*2}(\lambda) \subset \mathfrak{W}^2(\lambda)$, and $\mathfrak{Z}^{*2}(\lambda) = R_\lambda\{\mathfrak{M}[R_\lambda^{-1}(\mathfrak{k}^1(\lambda)); R_\lambda^{-1}(\mathfrak{W}^{*2}(\lambda))]\} \subset \mathfrak{Z}^2(\lambda)$.

(6) $\mathfrak{i}^{*3} = \underset{\lambda \epsilon \mathfrak{k}^1}{S} [\mathfrak{Z}^{*2}(\lambda) + \mathfrak{W}^{*2}(\lambda)]$ is intersected by another segment of an a.h.-s. $\mathfrak{p}^3 = \mathrm{E}[\psi(z_1, z_2, \mu) = 0]$ such that there exists a cell $\mathfrak{M}$, the boundary of which consists of parts of $\mathfrak{i}^{*3}$ and $\mathfrak{p}^{*3}$ (these parts denoted by $\mathfrak{i}^3$ and $\mathfrak{p}^3$, respectively).

(7) $\mathfrak{M}$ satisfies all the conditions formulated in [5].[7] Thus, every a.f. of 2 c.v. which is regular in $\mathfrak{M}$ can be represented by means of the integral formula (3.7) of [5][7] in terms of its values on $\mathfrak{F}^2 = \bar{\mathfrak{i}}^3 \cdot \bar{\mathfrak{p}}^3$.

(8) The domain $\underset{t_2 \epsilon \mathfrak{A}^2}{S} \mathfrak{M} \cdot (z_2 = t_2)$ (see [5],[7] p. 855) lies entirely in $\mathfrak{B}$.

THEOREM. *Suppose that an a.f. of 2 c.v. $w(z_1, z_2)$ which is regular in $\mathfrak{B}$ assumes on a regular intersection $\mathfrak{R}^2 = \underset{\lambda \epsilon \mathfrak{k}^1}{S} \mathfrak{k}^1(\lambda)$ of $\mathfrak{b}^3$ with a segment of an a.h.-s. values belonging to an arc of a circle. Then $w(z_1, z_2)$ can be analytically extended to $\mathfrak{M}$ where $\mathfrak{M}$ is the domain described above.*

Proof. By hypotheses is $W(\mathrm{Z}) = w[h^1(\mathrm{Z}, \lambda), h^2(\mathrm{Z}, \lambda)]$ regular in $R_\lambda^{-1}[\mathfrak{W}^{*2}(\lambda)]$, and it assumes values belonging to an arc of a circle on the analytic curve $R_\lambda^{-1}[\mathfrak{k}^1(\lambda)]$. Therefore, $W(\mathrm{Z})$ can be analytically extended in $R_\lambda^{-1}[\mathfrak{W}^{*2}(\lambda)]$. $w[h^1(\mathrm{Z}, \lambda), h^2(\mathrm{Z}, \lambda)]$ is an a.f. of 1 c.v. Z, regular in $R_\lambda^{-1}[\mathfrak{Z}^{*2}(\lambda) + \mathfrak{W}^{*2}(\lambda)]$.

Since $w[h^1(\mathrm{Z}, \lambda), h^2(\mathrm{Z}, \lambda)]$ can be extended in $\mathfrak{Z}^{*2}(\lambda)$ for every $\lambda(\lambda \epsilon \mathfrak{k}^1)$, $w(z_1, z_2)$ is defined in $\bar{\mathfrak{i}}^{*3}$, and therefore on $\mathfrak{F}^2 = \bar{\mathfrak{i}}^3 \cdot \bar{\mathfrak{p}}^3$. The expression

$$v(t_1, t_2) = (2\pi i)^{-2} \int_{\mathfrak{B}^2} \int w[\varphi_1(\lambda, \mu), \varphi_2(\lambda, \mu)] \cdot B(t_1, t_2, \lambda, \mu) \cdot \times [\Phi(t_1, t_2, \lambda) \cdot \Psi(t_1, t_2, \mu)]^{-1} d\lambda d\mu \quad (4.1)$$

defines a function in $\mathfrak{M}$ (see [5],[7] p. 860). We shall show:

1°. That $v(t_1, t_2)$ is an a.f. of 2 c.v. t_1, t_2, regular in $\mathfrak{M}$;

2°. That $v(t_1, t_2) = w(t_1, t_2)$.

Since $B(t_1, t_2, \lambda, \mu)/\Phi(t_1, t_2, \lambda) \cdot \Psi(t_1, t_2, \mu)$ is an a.f. of 2 c.v. t_1, t_2, regular in $\mathfrak{M}$, and since the integrand is continuous by (4) on $\mathfrak{F}^2$, 1°. follows directly from (4.1). On the other hand the integrand of (4.1) is equal to

$$(2\pi i)^{-1} \int_{\mathfrak{k}^{*1}} \{\mathfrak{i}[w(\varphi_1, \varphi_2); \mathfrak{b}^{*1}(\lambda)]/\Phi(t_1, t_2, \lambda)\} d\lambda \quad (4.2)$$

where $\mathfrak{i}[w(\varphi_1, \varphi_2); \mathfrak{b}^{*1}(\lambda)] =$
$= (2\pi i)^{-1} \int_{\mathfrak{b}^{*1}} w[h^1(\mathrm{Z}, \lambda), h^2(\mathrm{Z}, \lambda)] [D(h^1, h^2) / D(\lambda, \mathrm{Z})] \Phi[t_1, h^2(\mathrm{Z}, \lambda), \lambda] \times \{\Pi[h^k(\mathrm{Z}, \lambda) - t_k]^{-1}\} d\mathrm{Z}$

for $(t_1, t_2) \epsilon \bar{\mathfrak{T}}$. (See [5], p. 858.)

$\mathfrak{i}[w(\varphi_1, \varphi_2); \mathfrak{b}^{*1}(\lambda)]$ assumes values for every $t_2 \epsilon \mathfrak{A}^2$ given by the formula (2.5) of [5]. By this result and by the considerations of [5] it is clear that (4.2) assumes the values $w(t_1, t_2)$ for $(t_1, t_2) \epsilon \mathfrak{T}$. Therefore,

$w(t_1, t_2) = v(t_1, t_2)$ for $(t_1, t_2) \epsilon \mathfrak{T}$, and $w(t_1, t_2)$ can be analytically continued throughout $\mathfrak{M}$.

5. A procedure for analytical extension of PT's.

THEOREM. *Suppose the* PT W *given by*[8] $w_\rho = w_\rho(z_1, z_2)$ *transforms the domain* $\mathfrak{B}$ *into* $\mathfrak{G} = W(\mathfrak{B})$. *Suppose there exist two regular intersections* $\mathfrak{K}_\rho^2$ *of the boundary* $\mathfrak{b}^3$ *with the segment of an a.h.-s.* $\mathfrak{h}^3$ *which are transformed into surfaces lying in* $\mathrm{E}[|w_\rho - c_\rho| = C_\rho]$. *Also, let* $W[\mathfrak{p}_\rho{}^3]$ *omit* $\mathrm{E}[|w_\rho - c_\rho| \leqq n_\rho]$, $n_\rho < C_\rho$, *so that* $W[\mathfrak{W}_\rho{}^2(\lambda)]$ *lies in* $\Pi\mathrm{E}[n_\rho \leqq |w_\rho - c_\rho| \leqq C_\rho]$. *Suppose that* $W(\mathfrak{N})$, $\mathfrak{N} = \mathfrak{M}_1 \cdot \mathfrak{M}_2$, *is schlicht, and let* $\mathfrak{L}^2 = \mathrm{E}[w_\rho = w_\rho(u_1, u_2)]$ *be the image of* $\mathfrak{P}^2 = \mathrm{E}[z_\rho = z_\rho(u_1, u_2)] \subset \mathfrak{a}^3$, *i.e., let* $\mathfrak{L}^2 = W(\mathfrak{P}^2)$. *Then for the* B*-area of an element of* $\mathfrak{L}^2$ *we have the inequality*

$$\sqrt{\mathrm{K}_{\mathfrak{G}}(w, \bar{w})}\,|D(w_1, w_2)/D(u_1, u_2)|\,du_1 du_2 \leqq \sqrt{\mathrm{K}_{\mathfrak{D}}(z, \bar{z})}\,|D(z_1, z_2)/D(u_1, u_2)|\,du_1 du_2 \quad (5.1)$$

where $\mathfrak{D} = \mathfrak{B} + \mathfrak{N}$ *and* $\mathfrak{S} = \mathfrak{G} + \mathfrak{Q}$, $\mathfrak{Q} = \Pi\mathrm{E}[n_\rho \leqq |w_\rho - c_\rho| \leqq C_\rho{}^2/n_\rho]$.

Proof. Using the considerations of section 4 we conclude that $w_\rho(z_1, z_2)$ is regular in $\mathfrak{M}_\rho$. Since $W[\mathfrak{W}^2{}_\rho(\lambda)]$ lies in $\mathrm{E}[n_\rho \leqq |w_\rho - c_\rho| \leqq C_\rho]$, it follows that $W[\mathfrak{Z}^{*2}(\lambda)]$ lies in $\mathrm{E}[C_\rho \leqq |w_\rho - c_\rho| \leqq C_\rho{}^2/n_\rho]$. Hence $W(\mathfrak{N}) = \Pi W(\mathfrak{M}_\rho)$ lies in $\Pi\mathrm{E}[n_\rho \leqq |w_\rho - c_\rho| \leqq C_\rho{}^2/n_\rho]$. Since, by hypothesis, $\mathfrak{L}^2$ lies in $\mathfrak{a}^3$, $\mathfrak{L}^2$ lies in interior of $\mathfrak{B} + \mathfrak{a}^3 + \mathfrak{N}$. On the other hand, $W[\mathfrak{B} + \mathfrak{a}^3 + \mathfrak{N}] \subset \mathfrak{G} + \mathfrak{Q} = \mathfrak{S}$. Applying (2.2) we obtain (5.1).

[1] German letters denote manifolds, superscript the dimension of the manifold. We omit, however, the superscript when the manifold is four dimensional. $d\omega$ denotes the Euclidean area element in the case of a.f. of 1 c.v., and the volume element in the case of 2 c.v.

Non-Euclidean measures are measures invariant with respect to CT's and PT's, respectively. See, for details, [1] S. Bergman, "Sur les fonctions orthogonales de plusiers variables complexes avec les applications . . .," Interscience Publishers, New York, 1941.

[2] See [2], S. Bergman, *C. R. Acc. Sc. U. S. S. R.*, **16**, 11–14 (1937); [3] S. Bergman, *Rend. d. R. Acc. Naz. d. Lincei*, **19** (6a), 474–478 (1934) and [1].

[3] The quantities $\int_{\mathfrak{G}^2} K(z, \bar{z}) z^n \bar{z}^m d\omega$ and $\int_{\mathfrak{G}} K(z_1, z_2, \bar{z}_1, \bar{z}_2) z_1{}^n z_2{}^m \bar{z}_1{}^p \bar{z}_2{}^q d\omega$ are said to be the complex moments with the weighting function K of $\mathfrak{G}^2$ and $\mathfrak{G}$, respectively.

[4] Note that in $\lambda_{\mathfrak{S}}^{[2]}{}_{\mathfrak{G}^2}$ appear only complex moments of $\mathfrak{G}^2 \times \mathfrak{G}^2$ with the weighting function $\mathrm{K}_{\mathfrak{S}^2}$ which does not depend on $\mathfrak{G}^2$.

[5] $\Sigma = \sum_{\rho=1}^{\infty}$.

[6] See also [4] S. Bergman, *Bull. de l'Inst. math. Tomsk*, **1**, 242–257 (1937).

[7] [5] S. Bergman, *Recueil Math.*, **1**(**43**), 851–862 (1936).

[8] Since we deal now with two functions w_ρ, $\rho = 1, 2$, we shall add the subscript ρ to symbols introduced in section 4 in connection with the extension of $w(z_1, z_2)$. ρ will always assume values 1 and 2 in this section. Π will mean $\prod_{\rho=}^{2}$.